THE EPIDEMIOLOGY OF ARBOVIRUSES AND GASTROINTESTINAL PATHOGENS IN THE MIDDLE EAST AND NORTH AFRICA

A Thesis

Presented to the Faculty of the Weill Cornell Graduate School

of Medical Sciences

in Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

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May 2016

ABSTRACT

The focus of this thesis is to describe the epidemiology of two causes of infectious diseases that are relevant to the Middle East and North Africa (MENA): arboviruses and gastrointestinal pathogens. Manuscript 1 is entitled “The Epidemiology of Dengue, Chikungunya, and Yellow Fever in the Middle East and North Africa: Systematic Review and Meta-Analysis.” In this work, we reviewed published records across the MENA region to describe the human prevalence, human incidence, and vector infection rates for dengue (DENV), chikungunya (CHIKV), and yellow fever (YFV) viruses. Through a systematic search, we identified 145 human prevalence measures for the study viruses in the MENA region: DENV (n=104), CHIKV (n=24), and YFV (n=17). Seroprevalence measures >20% were identified for DENV (range 0-60%) and CHIKV (range 0-42%) in the Red Sea region and Pakistan, while evidence for YFV infections was confined to Sudan. Meta-analyses across all studies in the MENA region estimated general population mean IgG seroprevalence for DENV at 8.6% (95% CI: 5.3-12.6%), CHIKV 4.3% (95% CI: 1.1-9.1%), and YFV 3.7% (95% CI: 0.2-10.4%). However, there was considerable heterogeneity in effect size as well as high variability in subregional study coverage, target study populations, and diagnostic methods among reported studies. This study provides a detailed characterization of the epidemiology of these arboviruses in the MENA while identifying current research gaps and priorities for future research. Manuscript 2 is entitled “Multiplex PCR for Detection of Gastrointestinal Pathogens in Migrant Workers in Qatar.” In this work, we conducted a prospective observational study to understand the clinical characteristics and infectious causes of diarrhea among migrant

workers in Doha, Qatar. Seventy-five male workers coming to the Qatar Red Crescent Worker's Health Center outpatient clinic or emergency department were enrolled in the study. Surveys were administered to all subjects and the prevalence of 23 different stool pathogens was determined by multiplex PCR (FilmArray® Gastrointestinal PCR). Salmonella was the most prevalent pathogen and was detected in 27% of all subjects, followed by enteropathogenic E. coli (21%), enteroaggregative E. coli (15%) and enterotoxigenic E. coli (12%). In a multivariable analysis, a triage heart rate ≥ 90 beats per minute was the only significant predictor of a positive PCR result (OR 4.5, 95% CI 1.1-18.7). Use of multiplex PCR enabled the detection of gastrointestinal pathogens in 57% of cases overall, illustrating the utility of this diagnostic tool in epidemiologic studies of infectious diarrhea.

BIOGRAPHICAL SKETCH

John Moore Humphrey earned a Doctor of Medicine from the Medical School for International Health at Ben-Gurion University in Israel in 2009. He completed a combined residency in internal medicine and pediatrics at Tulane University School of Medicine from 2009 to 2013. He was named Resident of the Year in 2013. Following graduation from residency, he completed fellowship training in adult infectious diseases at Weill Cornell Medical College / New York-Presbyterian Hospital from 2013-2015. He is certified by the American Board of Internal Medicine (2013), American Board of Pediatrics (2014) and American Board of Internal Medicine - Infectious Diseases (2015). During his fellowship training in infectious diseases, Dr. Humphrey enrolled in the Master of Science in Clinical Epidemiology and Health Services Research in 2015 as part of an NIH T32 Pathogenesis of Infectious Diseases Training Program (PI Gulick, AI007613). The objective of this program is to train physician-scientists in basic and translational biomedical research focusing on the pathogenesis of infectious diseases. At the time of writing, Dr. Humphrey has first-authored five peer-reviewed publications indexed in Medline.

This work is dedicated to the migrant workers in Qatar

ACKNOWLEDGEMENTS

I would like to thank Dr. Marshall Glesby for his guidance and encouragement throughout this research project. His example as a scholar and educator is one that I hope to emulate, and I am fortunate to know him as a mentor and a friend. I would also like to thank Dr. Laith Abu-Raddad at Weill Cornell Medicine-Qatar. His mentorship and generosity facilitated a life experience that was for me both academically and personally enriching.

I would also like to thank the members of the Infectious Disease Epidemiology Group at Weill Cornell Medicine-Qatar, in particular Adona Canlas, for vital administrative and academic support throughout this project. I thank the clinical and laboratory staff of the Red Crescent Clinic, Ministry of Public Health, and Hamad Medical Center who participated in this study. I would also like to acknowledge funding support from the Infectious Disease Epidemiology Group at Weill Cornell Medicine-Qatar and NIH Research Training Grant T32 AI007613. Finally, I would like to thank the faculty in the Division of Infectious Diseases and the Masters Program in Clinical Epidemiology and Health Services Research. They have nurtured my interest in clinical research into a passion and vocation that I hope to pursue for years to come.

Above all, I owe my deepest gratitude to my wife, Connie. Her unwavering love and support as I pursue my career goals has been my greatest motivator.

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

LIST OF FIGURES

CHAPTER / MANUSCRIPT 1

CHAPTER / MANUSCRIPT 2

LIST OF TABLES

CHAPTER / MANUSCRIPT 1

CHAPTER / MANUSCRIPT 2

LIST OF SUPPLEMENTAL FIGURES, APPENDIX 1

LIST OF SUPPLEMENTAL TABLES, APPENDIX 1

LIST OF SUPPLEMENTAL TABLES, APPENDIX 1 CONTINUED

CHAPTER / MANUSCRIPT 1

TITLE PAGE

The Epidemiology of Dengue, Chikungunya, and Yellow Fever in the Middle East and North Africa: Systematic Review and Meta-Analysis

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ABSTRACT

The global distribution and disease burden of *Aedes*-borne arboviruses have increased in recent years. In the Middle East and North Africa (MENA) region, the epidemiology of dengue, chikungunya, and yellow fever remains poorly characterized. Following the PRISMA guidelines, we systematically reviewed available records across the MENA region describing the human prevalence, human incidence, and vector infection rates for dengue (DENV), chikungunya (CHIKV), and yellow fever (YFV) viruses. We synthesized the data in tables, maps, and meta-analyses estimating the region-specific prevalence for each virus in general populations and those with undifferentiated acute febrile illness (AFI) or 'suspected' dengue infection. In sum, we identified 145 human prevalence measures for the study viruses in the MENA region: DENV (n=104), CHIKV (n=24), and YFV (n=17). General and AFI-associated seroprevalence >20% was identified for DENV (range 0-60%) and CHIKV (range 0-42%) in the Red Sea region and Pakistan, while evidence for YFV infections was confined to Sudan. Meta-analyses across all studies in the MENA region estimated general population mean IgG seroprevalence for DENV at 8.6% (95% CI: 5.3-12.6%), CHIKV 4.3% (95% CI: 1.1-9.1%), and YFV 3.7% (95% CI: 0.2-10.4%). Among populations with AFI, mean prevalence estimates were DENV 18.6% (95% CI: 7.4-33.3%), CHIKV 8.8% (95% CI: 4.9-13.6%), and YFV 3.3% (95% CI: 0.0-22.4%). The meta-analyses identified considerable heterogeneity in effect size, and there was high variability in subregional study coverage, target study populations, and diagnostic methods among reported studies. Within the MENA region, available evidence suggests high DENV and CHIKV seroprevalence in the

Red Sea region and Pakistan, while YFV is confined to Sudan. However, coverage is incomplete and available evidence demonstrates high clinical and methodological heterogeneity. These findings illustrate a challenge to defining the geographic distribution and infection pressures of these viruses in the region and indicate priorities for future research.

INTRODUCTION

Like much of the world, the Middle East and North Africa (MENA) is experiencing an apparent surge in the spread of *Aedes*-borne arboviruses [1-6]. Over the past five years, reported dengue virus (DENV) infections reached unprecedented levels in the Arabian Peninsula and Pakistan [7, 8]. In 2011, local transmission of chikungunya virus (CHIKV) was recorded in the Arabian Peninsula for the first time during an outbreak in Yemen with over 15,000 suspected cases [9]. The following year, Sudan experienced a yellow fever virus (YFV) outbreak that was declared one of the worst outbreaks the world had seen in recent history [10]. In November 2015, DENV re-emerged in Egypt after a decades-long absence of reported cases from the country [11]. Although only snapshots, these reports raise critical questions about the unmeasured burden and distribution of *Aedes*-borne arboviruses in the MENA region and the risk that *Aedes*-borne pathogens pose to the region in the future [12, 13].

Still, gaining an understanding of the epidemiology of these viruses in the MENA region remains a challenge [14, 15]. Inadequate human and vector surveillance, non-reporting, and poor diagnostic capacity limit arbovirus

detection in many MENA countries, resulting in delays in outbreak recognition and sparse data with which to estimate disease burden and infection rates [9, 12, 13]. In the absence of such capacity, differentiating between the various arboviral causes of acute febrile illness (AFI) by clinical grounds alone is often unreliable [9, 16-19], with reports of CHIK being mistaken for both YF [20] and DEN [9, 17], DEN being mistaken for Crimean-Congo Hemorrhagic Fever (CCHF) [21], and CCHF being mistaken for DEN [18, 19]. Even with laboratory capacity, the well-known cross-reactivity of flavivirus antibodies in serological diagnostic tests adds another layer of uncertainty to both clinical decision making and epidemiologic research [22, 23].

To move beyond the uncertainties surrounding the epidemiology of *Aedes*-borne arboviruses in the MENA, we present a comprehensive summary and appraisal of published prevalence, incidence, and outbreak data for DENV, CHIKV, and YFV in the region. These arboviruses were selected because of their putative emerging presence in the MENA and because of their shared mosquito vectors, *Aedes aegypti* and *Aedes albopictus*, which facilitate the potential for these viruses to have overlapping geographic distribution. Applying a systematic approach, we aimed to 1) provide an in-depth summary of the peer-reviewed literature detailing the prevalence and incidence of these viruses in humans, their infection rates in vectors, and reported human outbreaks, 2) summarize human prevalence data from a meta-analytic approach, and 3) describe epidemiologic knowledge gaps and areas at risk of *Aedes*-transmitted arboviruses. This report will enhance the understanding of the epidemiology of DENV, CHIKV, and YFV in the MENA and inform future research priorities

MATERIALS AND METHODS

Data Sources and Search Strategy. We conducted separate systematic reviews of the prevalence and incidence of DENV, CHIKV, and YFV in the MENA following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [24]. The PRISMA checklist is found in S1 Figure and our search criteria in S2 Figure. Briefly, we searched PubMed, Embase, the World Health Organization (WHO) Index Medicus for the Eastern Mediterranean Region (EMRO) and WHO African Index Medicus (AIM) (up to December 9, 2015) using separate search criteria for each virus without publication date or language restrictions, using text and MeSH/Emtree terms exploded to include all subheadings. Our review covered the 23 countries included in the MENA definitions of the WHO/EMRO, World Bank, and the Joint United Nations Programme on HIV/AIDS (UNAIDS) for consistency with earlier regional analyses of various infections diseases including HIV (S3 Figure)[25].

Study Selection. For each study virus, titles and abstracts were imported into Endnote (Thompson Reuters, Philadelphia, PA, USA), duplicates removed, and screened by one author (JH) with potential eligibility determined by consensus with a second author (NC) when necessary. Full texts of potentially relevant records were retrieved and assessed for eligibility. Reference lists of all potentially eligible articles and reviews were also searched. Studies containing primary prevalence or incidence data pertaining to at least one of the study viruses in the MENA region were considered eligible for the systematic review (Figure 1.1). Case reports, case series, editorials, letters to

editors, reviews, commentaries, qualitative studies, basic science research studies, and studies from countries outside the region were excluded from the systematic review. One YFV seroprevalence study conducted among YFV vaccine recipients was also excluded from the YFV review given the inability to differentiate wild and vaccine-derived antibodies [26]). In our study, the term ‘report’ refers to the document (paper, abstract, or public health record) containing an outcome measure of interest, while ‘study’ refers to the outcome measure(s) within that report. Hence, reports could contribute more than one study, although multiple reports of the same study were counted only once. Outbreak reports were identified through a separate search using the same search criteria. Outbreaks were defined by the author’s description of the event as an outbreak. Multiple reports of the same outbreak were counted only once.

Figure 1.1 PRISMA flow diagrams of article selection

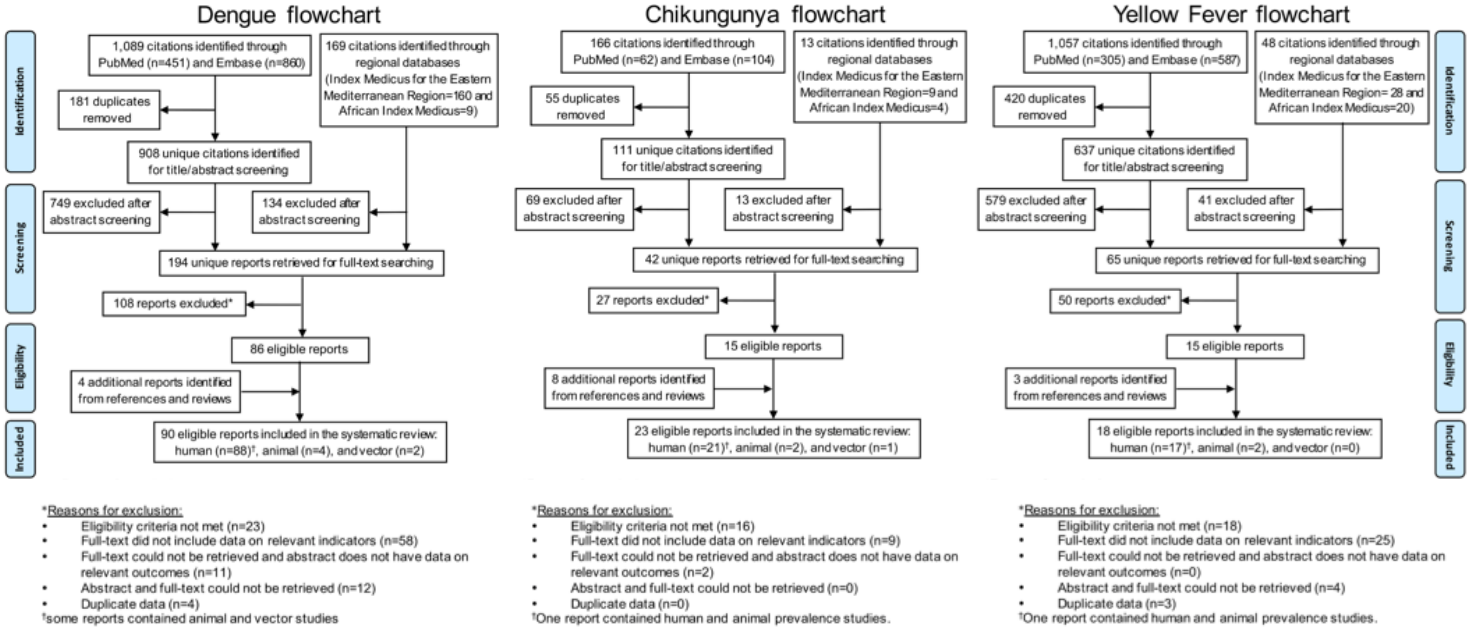

Figure 1.1 PRISMA flow diagrams of article selection. Flow diagrams for dengue, chikungunya, and yellow fever viruses in the Middle East and North Africa according to PRISMA guidelines.

Data Extraction and Synthesis. Data were extracted by one of the authors (JH) using a pre-piloted data extraction form and entered into a database created in Microsoft Access. Data from reports in English were extracted from the full texts, while reports in French (n=3), Turkish (n=3) and German (n=1) were extracted from the English abstract and with the help of online language software [27]. There were no records in other languages. The list of extracted information is located in S4 Figure. For each virus, studies were synthesized by country and organized by year, using separate tables for human and animal prevalence and vector infection rates (i.e. the estimated proportion of infected mosquitoes in a population at a given time). Human prevalence studies were further separated into three groups: general seroprevalence studies, undifferentiated acute febrile illness (AFI) studies, and studies of 'suspected' dengue infection. The definitions for these populations are found in S5 Figure. We also appraised the quality of the general population and AFI population prevalence measures by assessing the risk of bias (ROB) for each study using an adaptation of the Cochrane approach and by evaluating the precision of the reported measures [28]. A description of the quality assessment criteria and ROB assessment is found in S6 Figure. Finally, we mapped the geographic distribution of all human prevalence studies and at the level of the province/governorate in the MENA region (Tableau Software, Seattle, WA, USA). We manually marked the location of reported outbreaks on these maps as well, designating one mark per province in which one or more outbreaks were reported.

Quantitative analysis. Meta-analyses of human prevalence studies among general populations were conducted for each virus using the most specific IgG

serologic measure reported for each study (e.g. viral neutralization test (VNT) rather than ELISA, when available). Meta-analyses of the seroprevalence among populations with acute undifferentiated febrile illness (AFI) populations were also conducted for each virus, utilizing baseline IgG seroprevalence measures for the study population, which were more likely indicative of secondary, rather than primary, infection. Studies that did not include baseline IgG seroprevalence measures, including studies of 'suspected' dengue infection and some AFI studies, were not included in this analysis.

The variance of the prevalence measures was stabilized using the Freeman-Tukey type arcsine square-root transformation [29]. Prevalence estimates were weighed using the inverse variance method and then pooled using a DerSimonian-Laird random effects model [30]. This model assumes a normal distribution of true effect sizes across studies, and therefore accounts for the sampling variation as well as the heterogeneity in effect size [31]. The I^2 heterogeneity measure and its confidence interval (CI) were calculated to assess the magnitude of between-study variation that is due to differences in effect size across studies rather than chance [32]. Other heterogeneity measures were also calculated, including Cochran's Q statistic to test for heterogeneity in effect size, τ^2 to estimate the between-study variance of the true effect size, and prediction intervals to estimate the 95% interval in which the true effect size in a new study will lie [31, 33].

Finally, a meta-regression was performed to identify between-study heterogeneity in the pooled mean general population prevalence estimates for DENV [28, 34]. The following covariates were examined in univariable and

multivariable models: country, year of study, assay type (ELISA, complement fixation, hemagluttinin inhibition, immunoflouresence antibody test, or viral neutralization titer), sampling method, and the precision of the measure (i.e. ≥100 participants). These covariates were included in the multivariable model if the p-value was <0.1.

RESULTS

Search Results. The selection processes based on PRISMA guidelines are illustrated for each of the study viruses in Figure 1.1 [24]. Briefly, the DENV search yielded 1,258 citations, 90 of which were ultimately eligible for inclusion in the study with the addition of 4 reports identified from the bibliographies of relevant reports and reviews. The CHIKV search yielded 179 citations, 23 of which were eligible for inclusion in the study following screening process and after the addition of 8 reports. For YFV, 1,105 citations were retrieved through a separate search, 18 of which were eligible following the screening process and with the addition of 3 external reports.

Dengue Overview. A total of 104 human prevalence studies for DENV were identified from eligible reports (Table 1.1). These studies covered 13 of 24 MENA countries and were conducted from 1962-2015. The geographic distribution of these studies is illustrated in Figure 1.2. Anti-DENV antibodies were reported from 12 of 13 countries in which studies were reported with a single 1973 study from Libya reporting 0% seroprevalence [35]. The highest number of studies were reported from Pakistan (n=30) and Sudan (n=16), most of which targeted populations with AFI or those with suspected dengue

infection (Table 1.1). Among general populations, IgG prevalence measures ranged from 0% to 61% and were reported from Djibouti (n=4, 0-21%), Egypt (n=4, 0-7%), Iran (n=3, 0-7%), Kuwait (n=3, 0-56%), Lebanon (n=3, 0-61%), Pakistan (n=3, 9-28%), Saudi Arabia (n=4, 0-33%), and Sudan (n=5, 9-49%). However, observed or potential serologic cross-reactions with other flaviviruses were present in multiple studies, while the results of VNT were reported in 3% (n=3) of studies [26, 36, 37]. Three human incidence measures for DENV were identified (S1 Table); the first reported an ELISA IgM incidence of 35 cases per 10,000 people living in urban homes where DENV-carrying mosquitoes were identified [38]; the second reported an ELISA IgM incidence of 94 cases per 10,000 people among a general population in Port Sudan, Sudan over a 17 week period in 2010 [39]; the third reported an ELISA IgM, NS1 antigen, or PCR incidence of 18 cases per 10,000 people among febrile children in an urban slum in Karachi, Pakistan from 1999-2001 [40]. Four reports containing DENV seroprevalence measures in animals from the 1970-80s were identified from Pakistan, Tunisia, and Turkey but may also have represented cross-reactions with other flaviviruses (S2 Table) [41-44]. Two reports containing DENV infection rates in *A. aegypti* populations were identified in Pakistan and Yemen, respectively (S3 Table).

Table 1.1 Summary of human prevalence studies for dengue virus in the Middle East and North Africa (n=104).

Table 1.1

Country, Ref.	Year(s) of study[e]	City or governorate	Setting; population (age range, years)	Sampling	Assay type	Assay make[†]	Target Protein	Assay serotype	Sample size	Prevalence	Additional testing & Comments
Afghanistan (n=1)											
Elyan [45]	2008-10	Uruzgon, Helmand, Kandahar	Hospital; AFI patients (20-59)	Conv.	ELISA	PanBio	Env	1-4	913	**19.2%****	2.6% (8/312) were IgM+; observed cross-reaction to WNV, TBEV
Djibouti (n=6)											
Salah [46]	1987	Djibouti City	Military; healthy soldiers	Conv.	IIFA	In-house	wv	2	50	**0%**	
		Randa	Rural community; general pop.	Conv.	IIFA	In-house	wv	2	69	**0%**	
		Djibouti City	Hospital; AFI patients	Conv.	IIFA	In-house	wv	2	41	**0%**	
Rodier [37]	1991	Djibouti City	Clinical setting; AFI patients (1-55)	Conv.	ELISA IgM	In-house	wv	1	91	**7.7%****	3.7% (1/27) were VNT+; multiple observed cross-reactions
				Conv.	ELISA IgM	In-house	wv	2	*same*	**25.2%****	11.1% (3/27) were VNT+; multiple observed cross-reactions
				Conv.	ELISA IgM	In-house	wv	3	*same*	**16.4%****	multiple observed cross-reactions
				Conv.	ELISA IgM	In-house	wv	4	*same*	**18.7%****	multiple observed cross-reactions
Fauld [47]	2011	Djibouti City	Animal quarantine station; workers	Conv.	IIFA	EuroImmun	wv	1-4	10	**10.0%****	not cross-reactive to WNV
Andayi [48]	2010-11	Djibouti City	Community; general pop. (<1-100)	SRS	ELISA	PanBio	Env	1-4	911	**21.8%**	
Egypt (n=5)											
Mohammed [49]	1966	Abyss	rural community; general pop.	Conv.	HI	In-house	wv	1	29	**7.0%****	possible cross-reaction to WNV
					HI	In-house	wv	4	*same*	**3.0%****	possible cross-reaction to WNV
		Alexandria	urban community; general pop.	Conv.	HI	In-house	wv	1	55	**4.0%****	possible cross-reaction to WNV
					HI	In-house	wv	4	*same*	**5.0%****	possible cross-reaction to WNV
Mohammed [50]	1968	Alexandria	Hospital; AFI patients (3-13)	Conv.	HI, CF	In-house	wv	1	120	**0%****	0% (0/48) were convalescent +
		Alexandria	Clinical setting; adults	Conv.	HI	In-house	wv	1	78	**0%**	
Darwish [51]	1969	Multiple	University; students	Conv.	HI	In-house	wv	1	1133	**0.3%**	
Iran (n=4)											
Saidi [52]	1970	Multiple	n/s	n/s	HI	In-house	wv	1,2,3	394	**6.0%****	possible cross-reaction to WNV
Saidi [53]	1970-71	Caspian region	Community; children (1-6)	Conv.	HI	In-house	wv	2	100	**0%**	
Chinikar [54]	2000-12	Countrywide	Clinical setting; AFI patients	Conv.	ELISA	Vircell	wv	1,2	300	**3.3%**	3.3% (10/300) were IgM+; DEN-1,2 were positive by PCR
Aghaie [13]	2014	Sistan-Baluchestan	blood donor center; general pop.	Conv.	ELISA	PanBio	Env	1-4	540	**7.6%**	78% (32/41) ELISA+ were IFA+
Kuwait (n=8)											
Ibrahim [55]	1966-68	Multiple	Multiple settings; blood donors, non-AFI patients, children (1-60)	Conv.	HI	In-house	wv	1	627	**6.5%****	not cross-reactive to DEN-2 or WNV
					HI	In-house	wv	2	*same*	**8.1%****	not cross-reactive to DEN-1 or WNV
Al-Nakib [56]	1979-82	Jabriya	Hospital; non-AFI patients (0-60+)	SRS	HI	In-house	wv	1	502	**3.2%****	not cross-reactive to DEN-2 or WNV
					HI	In-house	wv	2	*same*	**8.4%****	all were cross-reactive to DEN-1, WNV, or TBEV
Pacsa [57]	2002*	1997-99	n/s; Kuwaiti nationals	n/s	ELISA and IgG blot	CDC and Genlab	wv	1-4	425	**13.9%**	only DENV 1-3 were positive

Table 1.1 Continued

			n/s; Kuwait Bedouins	n/s	ELISA and IgG blot	CDC and Genlab	wv	1-4	47	0%	
			n/s; expatriates from South Asia	n/s	ELISA and IgG blot	CDC and Genlab	wv	1-4	266	37%	only DENV 1-3 were positive
			n/s; expatriates from Southeast Asia	n/s	ELISA and IgG blot	CDC and Genlab	wv	1-4	31	56.6%	only DENV 1-3 were positive
			n/s; expatriates from Middle East	n/s	ELISA and IgG blot	CDC and Genlab	wv	1-4	140	25%	only DENV 1-3 were positive
			Hospital; returned travelers with dengue-like illness	n/s	ELISA IgM	PanBio	Env	1-4	210	9.0%	only DENV 1-3 were positive; 10%(2/19) IgM+ were PCR+
Lebanon (n=3)											
Garabedian [58]	1962-65	Multiple	Community; general pop. (0-41+)	SRS	HI	In-house	wv	2	113	61.9%**	observed cross-reaction with WNV, YFV
		Multiple	Community; general pop. (0-41+)	SRS	HI	In-house	wv	1	171	49.1%**	observed cross-reaction with WNV, YFV
Hatem [59]	1969	Beirut	n/s	n/s	HI	In-house	wv	2	126	0%	
			n/s	n/s	HI	In-house	wv	1	*same*	4.0%**	observed cross-reaction with WNV
Libya (n=1)											
Darwish [35]	1973	Sebha	community and clinic; children, non-AFI patients	Conv.	HI	In-house	wv	1	148	0%	
Pakistan (n=30)											
Darwish [41]	1983*	Karachi	Hospital; patients	Conv.	CF	In-house	wv	1	43	9.3%	
Akram [60]	1994	Karachi	Hospital; AFI patients (<1-12)	Conv.	ELISA IgM	In-house	wv	1	92	9.8%**	12% (3/25) additional convalescent sera were +; observed cross-reaction to WNV
				Conv.	ELISA IgM	In-house	wv	2	*Same*	14.6%**	24% (6/25) additional convalescent sera were +; observed cross-reaction to WNV
Siddiqui [40]	1999-2001	Karachi	Community; AFI patients (<16)	Conv.	ELISA IgM	Diag. Auto.	wv	1-4	341	15.8%	
Tariq [61]	2003	Mangla, Mirpur	Community; suspected dengue	Conv.	ELISA IgM	In-house	n/s	n/s	52	73%	
Jamil [62]	2005	Karachi	Hospitals; suspected dengue	Conv.	ELISA IgM	Chemicon	n/s	n/s	106	36.8%	
Khan [63]	2006	Karachi	Hospital; suspected dengue (2-72)	Conv.	ELISA IgM	PanBio	Env	1-4	83	83.6%	
				Conv	ELISA IgM	Calbiotech	PA	1-4	*same*	50.7%	87.8% (73/83) were PCR+ for DEN-2,3 only
Khan [64]	2006	Karachi	Hospital; suspected dengue	Conv.	ELISA	PanBio	Env	1-4	250	23.2%	53.6% (134/250) were IgM+; 74% (185/250) were PCR+ for DEN-2 or 3
Koo [65]	2006-11	Multiple	Clinic settings; suspected dengue	Conv.	PCR	In-house		2,3	200	47%	none were DEN-1 positive
Khan [66]	2006-07	Hyderabad	Hospital; suspected dengue (13-70)	Conv.	ELISA IgM	In-house	n/s	n/s	50	40%	
Khan [67]	2006-07	Multiple	Hospital; suspected dengue	Conv.	ELISA IgM	Calbiotech	PA	1-4	15,040	26.3%	
Abbasi [68]	2007-08	Karachi	Hospital; suspected dengue	Conv.	ELISA IgM	Commercial	n/s	n/s	114	69.6%	
Tahir [69]	2008	Lahore	Hospital; suspected dengue	Conv.	ICT (IgM)	In-house	n/s	n/s	3215	54.9%	
Murad [70]	2008	Shangla	Community; suspected dengue (1-80)	Conv.	ELISA IgM	n/s	n/s	n/s	70	17.1%	

Table 1.1 Continued

Kidwai [71]	2008-09	Karachi	Hospital; suspected dengue (>13)	Conv.	ICT (IgG)	In-house	wv	1-4	599	**83.2%**	41.9% (251/599) were IgM+
Zafar [72]	2009	Rawalpindi	rural communities; adults without history of flavivirus vaccination (>18)	StRS	ELISA	Omega	PA (DEN-2)	1-4	96	**19.8%**	
Zafar [73]	2009	Rawalpindi	Community; general pop.	Conv.	ELISA	Omega, Vircell	PA (DEN-2)	1-4	244	**28.8%**	
Qureshi [74]	2010-12	Karachi	Hospital; suspected dengue	Conv.	ICT (IgM)	In-house	n/s	n/s	162	**9.9%**	
Khan [75]	2010	Punjab	Hospital; suspected dengue (4-60)	Conv.	ELISA IgM	n/s	n/s	n/s	125	**54.4%**	
Hasan [76]	2010	Karachi	Hospital; suspected dengue (>12)	Conv.	ELISA IgM	n/s	n/s	n/s	259	**34.8%**	
Umar [77]	2010	Rawalpindi	Hospital; suspected dengue	Conv.	ELISA IgM	n/s	n/s	n/s	500	**6.8%**	
Jameel [78]	2010	Lahore	Hospital; suspected dengue	Conv.	ELISA IgM	In-house	n/s	n/s	341	**48.7%**	
Naeem [79]	2011	Lahore	Hospital; AFI patients (1-10+)	Conv.	ELISA IgM	n/s	n/s	n/s	79	**25.3%**	
Ahmed [80]	2011	Lahore	Hospital; suspected dengue (13-81)	Conv.	ELISA IgM	n/s	n/s	n/s	640	**43.9%**	
Ijaz [81]	2011	Lahore	Hospital; suspected dengue (<15-60+)	Conv.	ELISA	n/s	n/s	1-4	5,274	**49%**	
Rashid [82]	2011	Lahore	Hospital; suspected dengue (<18)	Conv.	ELISA	n/s	n/s	n/s	254	**36.6%**	53.9% (137/254) were IgM+
Khan [83]	2011	Lahore	Hospital; suspected dengue (5-50+)	Conv.	ELISA	In-house	wv	1-4	50	**72%**	30% (30/50) were IgM+; 66% (33/50) were PCR+ for DEN-1,2; 60% (30/50) were cell culture+
Hasan [84]	2007-13	Multiple	Hospitals; suspected Crimean-Congo Hemorrhagic Fever	Conv.	ELISA IgM	PanBio	Env	1-4	168	**33.9%**	2.3% (4/168) were PCR+
Ali [85]	2011	Khyber Pakhtunkhwa	Clinical settings; suspected dengue (<10 to >51)	Conv.	ELISA	Diag. Auto.	wv	1-4	612	**20.2%**	31.9% (195/612) were IgM+
Hisam [86]	2012	Rawalpindi	Military Hospital; AFI patients	PS	ELISA IgM	n/s	n/s	n/s	500	**3.2%**	
Assir [87]	2012	Lahore	Hospital; suspected dengue (12-90)	Conv.	ELISA IgM	GmbH	wv	1-4	85	**43.5%**	20% (3/15) were PCR + for DEN-2
Saudi Arabia (n=11)											
Fakeeh [88]	1994-99	Jeddah	Hospitals; suspected dengue (1->50)	Conv.	IIFA, HI	In-house	wv	1,2	985	**31.9%**	16.2% (160/985) were ELISA IgM+; 21% (207/985) were PCR+ (DEN-1,2,3)
Fakeeh [89]	1994-2002	Jeddah	Hospitals; suspected dengue	Conv.	IFA, HI	In-house	wv	1,2,3	1020	**50.5%**	10.8% (110/1020) were ELISA IgM+; 20.5% (209/1020) were PCR+ (DEN-1,2,3)
Khan [90]	2004	Makkah	Hospital; suspected dengue (6-94)		ELISA	PanBio	Env	1-4	136	**32.4%**	58.8% (80/136) were IgM+; 28.1% (27/96) were PCR + (DEN-2,3)
Ayyub [91]	2004-05	Jeddah	Hospital; suspected dengue (2-60)	Conv.	ELISA IgM	n/s	n/s	n/s	80	**48.8%**	
Shahin [92]	2006-08	Makkah	Hospital; suspected dengue	Conv.	ELISA IgM and/or PCR	n/s	n/s	n/s	159	**100%**	
Said [93]	2006	Jeddah	Hospital; suspected dengue (2-71)	Conv.	ELISA IgM	In-house	n/s	n/s	525	**19.2%**	% includes paired serum sample
Memish [94]	2010	Multiple	Military; adults	Conv.	ELISA	PanBio	Env	1-4	1024	**0.1%**	0% of IgG+ were IgM+
Gamil [95]	2010-11	Jeddah	Hospitals; suspected dengue (3-56)	Conv.	n/s	n/s	n/s	n/s	553	**47.7%**	
Al-Azraqi [96]	2013	Jizan	Clinics; clinic attendants (1-60+)	SRS	ELISA	Focus	wv	1-4	268	**26.5%**	
		Aseer	Clinics; clinic attendants (1-60+)	SRS	ELISA	Focus	wv	1-4	697	**33.7%**	

Table 1.1 Continued

Ashshi [97]	2014	Mecca	blood donation center; adults	Conv.	ELISA	PanBio	Env	1-4	100	7%	6% (6/100) were IgM+; 1% (1/100) were NS1+
Somalia (n=7)											
Botros [98]	1987	Hargeysa	Refugee camp; AFI patients	Conv.	ELISA	In-house	wv	2	28	**60.7%**	acute and convalescent samples 39.4% (15/38) were IFA+ 37.9% (11/29) were HI+ 14.2% (4/28) were ELISA IgM+
Kanesa [99]	1993	n/s	Military base; AFI soldiers	Conv.	ELISA IgM and/or HI	n/s	n/s	n/s	84	**17.8%**	93% (14/1) were cell culture + (DEN-2 and 3 only)
Sharp [100]	1992-93	Mogadishu	Military Hospital; AFI patients (soldiers)	Conv.	ELISA IgM	In-house	wv	1-4	129	**34.9%**	40.6% (39/96) were cell culture positive for DEN-2; 2% (2/96) were cell culture positive for DEN-3
		Baardera	Military; adults (19-25)	Conv.	ELISA IgM	In-house	wv	1-4	494	**7.7%****	observed cross-reaction with WNV
Nur [101]	1995	Mogadishu	Hospital; children (<1 to > 2 years of age)	CC.	ELISA IgM	Progen	wv	2	23	**0%**	
			Hospital; AFI patients with / without rash (<1 to > 2 years of age)	CC.	ELISA IgM	Progen	wv	2	46	**0%**	
Kyobe Bosa [102]	2011	Mogadishu	Hospitals; AFI patients (20-49)	Conv.	ELISA IgM	n/s	n/s	1,2,3	134	**80%**	62% (83/134) were PCR+
Sudan (n=16)											
Omer [36]	1976	Gezira	Rural community; general pop. (5-40+)	Conv.	HI	In-house	wv	2	109	**27.5%**	17.4% (19/109) were VNT+
Hyams [103]	1984	Port Sudan	Hospital; AFI patients (12-70)	Conv.	HI	In-house	wv	n/s	100	**3%**	14.8% (8/54) were convalescent + 1% (1/100) DEN-1 cell culture + 17% (17/100) DEN-2 cell culture +
Woodruff [104]	1986	Juba	Hospital; patients with history of fever within past 6 months and AFI patients (1-85)	Conv.	HI	In-house	n/s	n/s	130	**40.0%****	represents single virus activity not cross-reactive to multiple flaviviruses tested
McCarthy [105]	1988	Khartoum	Clinical setting; non-AFI patients	CC	ELISA	In-house	wv	2	100	**49%**	0% were IgM+
			Clinical setting; AFI patients (1-89)	CC	ELISA	In-house	wv	2	196	**48%****	0% were IgM+; possible cross-reaction to WNV
Watts [19]	1989	Northern Province	Clinical setting; AFI patients (11-70)	Conv.	ELISA	In-house	n/s	2	185	**24.0%****	possible cross-reactions to multiple flaviviruses
Ibrahim [106]	1997-99	Khartoum	Clinical setting: suspected measles	Conv.	ELISA IgM	MRL Diag.	n/s	n/s	188	**3.2%**	
Malik [107]	2004-05	Port Sudan	Hospitals; suspected dengue (<1-15)	Conv.	ELISA IgM	PanBio	Env	1-4	40	**90.0%**	39% (9/23) were PCR+ (DEN-3)
Gould [20]	2005	South Kordofan	Clinical setting; suspected YF patients (n=3), severe illness (n=8), AFI patients (n=7), healthy (n=16)	Conv.	ELISA IgM	In-house	wv	n/s	34	**5.9%****	observed cross-reaction with YFV, WNV
Farnon [26]	2005	Kortalla	Community; general pop., YF vaccinated (0-44+)	SSCS	ELISA	In-house	wv	1-4	84	**1.1%****	observed cross-reaction in YF vaccine recipient; 0% were IgM+
Seidahmed [38]	2008-09	Port Sudan City	Urban community; individuals from houses with DENV-carrying mosquitoes (<1-80)	RSS	ELISA IgM	PanBio	Env	1-4	791	**5.2%**	

Table 1.1 Continued

Adam [108]	2008-09	Port Sudan City	Hospitals; pregnant women with deliveries	Ret. cohort	ELISA IgM	n/s	n/s	1-4	10,820	**0.7%**	
Himatt [109]	2011	Kassala state	Community; general pop. (5-75+)	MSCS	ELISA	PanBio	Env	1-4	489	**9.4%**	0.6% (3/489) were IgM+
Abdalla [110]	2012	Kassala State	Hospital; AFI patients with suspected measles (2-65)	Conv.	ELISA	PanBio	Env	1-4	60	**11.7%**	
Elduma [16]	2012	Port Sudan	Hospital; pregnant women with AFI	Conv.	ELISA	Commercial	n/s	n/s	39	**12.8%**	2.6% (1/39) were IgM+ and PCR+
Soghaier [111]	2014	South Kordofan	Urban and rural communities; general pop. (15-60)	MSCS	ELISA	PanBio	Env	1-4	600	**27.7%**	77% of study population were YFV vaccinated
Turkey (n=6)											
Ari [44]	1971	Izmir	Community and clinic; general pop.	Conv.	HI	In-house	wv	2	270	**0%**	
Radda [112]	1973*	Izmir	n/s; general pop.	Conv.	HI	In-house	wv	2	270	**0.3%****	observed cross-reaction with WNV
		Istanbul	n/s; general pop.	Conv.	HI	In-house	wv	2	90	**0%**	
		Ankara	n/s; general pop.	Conv.	HI	In-house	wv	2	95	**0%**	
Ergunay [113]	2010	Ankara, Konya, Eskisehir, Zonguldak	blood donation center; blood donors	Conv.	ELISA	EuroImmun	wv	1-4	2435	**0.9%**	14.2% (3/21) of IgG+ were IIFT+ for DEN-2; 9.5% (2/21) of IgG+ were IgM+
Tezcan [114]	2010-11	Mersin	blood donation center; blood donors	Conv.	ELISA	Vircell	wv	1-4	920	**16.6%**	0.9% (8/920) were IgM+; 0% were NS1+
Yemen (n=5)											
Bin Ghouth [115]	2011	Hadramout	Hospital; suspected dengue (<5 to 55+)	Conv.	ELISA	PanBio	Env	1-4	982	**50.6%**	64.1% (630/982) IgM+ 86.2% (163/189) PCR+ for DEN-3
Malik [116]	2010-11	Al-Hudaydah	Clinical setting; AFI patients (0-45+)	Conv.	ELISA	PanBio	Env	1-4	136	**87.5%**	8.1% (11/136) were IgM+
Madani [117]	2010	Hadramout	Clinical settings; suspected viral hemorrhagic fever (3-75)	Conv.	ELISA	PanBio	Env	1-4	207	**48.3%**	78.7% (163/207) IgM+; 46.9% (97/207) NS1+; 0.09% (2/207) PCR+ for DEN-1,2
Rezza [118]	2012	Al Hudaydah	Hospitals; AFI patients with dengue-like illness (1-60)	CS	ELISA	NovaLisa	Env	1-4	*same*	**72.5%**	18% (72/400) IgM+; 13.8% (55/400) PCR+ for DEN-1,2
Qassem [119]	2013	Hadramout	Clinical setting; suspected dengue and/or west nile infection	Conv.	ELISA IgM	n/s	n/s	n/s	42	**19.0%****	observed cross-reaction with WNV

* Indicates year of publication when year(s) of data collection not available in report.

† All serologic assays were IgG unless otherwise stated.

**Indicates documented occurrence or suspicion of false-positives due to cross-reactions with other same family viruses or low serologic titers.

Abbreviations: AFI, acute febrile illness patients; Ag, antigen; CF, complement fixation; Conv, convenience; ELISA, enzyme-linked immunosorbent assay; HI, hemagglutinin inhibition; ICT, immunochromatography test; IIFT, indirect immunofluorescence test; MSCS, multi-stage cluster sampling; n/s, not specified; NS1, NS1 antigen test; PA, purified antigen; PCR, polymerase chain reaction; pop., population; PS, purposive sampling; RSS, random stratified sampling; SRS, simple random sampling; SSCS, single stage cluster sampling; VNT, viral neutralization test

Assay Abbreviation: CDC (Centers for Disease Control and Prevention, USA); Chemicon (Chemicon, Temecula, CA, USA); Diag. Auto. (Diagnostic Automation, CA, USA); Euroimmun (Lubeck, Germany); Focus (Focus Diagnostics, Cypress CA, USA); Genlab (Genlab Diagnostics, Singapore); GmbH (Human GmbH, Wiesbaden, Germany); MRL Diagnostics (Cypress CA, USA); NovaLisa (Dietzenbach, Germany); Omega (Omega Diagnostics, Scotland, UK); PanBio (Brisbane, Australia); Progen (Heidelberg, Germany); SD Bioline (Standard Diagnostics, Korea); Vircell (Vircell Microbiologists, Granada, Spain)

Figure 1.2 Geographic distribution of human prevalence studies and reported outbreaks for dengue, chikungunya, and yellow fever viruses in the Middle East and North Africa.

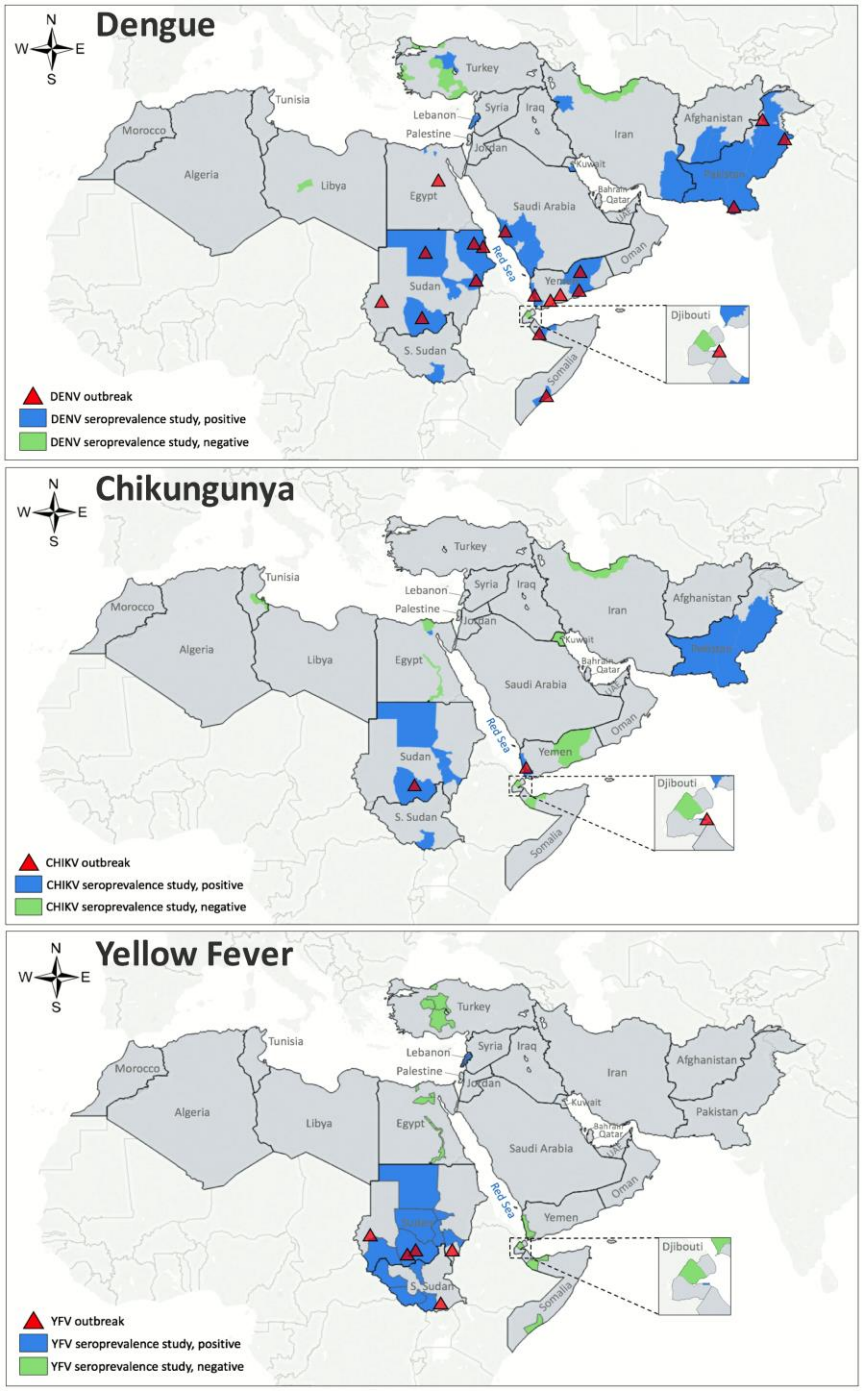

Figure 1.2 Geographic distribution of human prevalence studies and reported outbreaks for dengue, chikungunya, and yellow fever viruses in the Middle East and North Africa.

Chikungunya Overview. A total of 24 human seroprevalence studies for CHIKV were identified from eligible reports, 72% of which were conducted prior to 1990 (Table 1.2). Figure 1.2 illustrates the geographic distribution of CHIKV prevalence studies in the MENA region. Overall, Sudan reported both the highest number of human seroprevalence studies (n=8) and the highest general population seroprevalence (range 12-43%) in the region. High seroprevalence among febrile patients was identified in Yemen, in which three studies following identification of a CHIK outbreak reported ELISA IgM or RT-PCR prevalence among febrile patients of 9-28% [116-118]. Additional positive general or AFI seroprevalence measures were reported from Djibouti, Egypt, Iran, Kuwait, and Pakistan. However, serologic cross-reactions with related alphaviruses (O'nyong-nyong, Sandfly Fever, and Sindbis viruses) or travel-acquired infections were observed or could not be excluded in some studies from Djibouti [46], Iran [52], Kuwait [56, 120], and Pakistan [41]. VNT were utilized in a total of three studies from Djibouti [48] and Sudan [36, 121], confirming the presence of anti-CHIKV antibodies in general popoulations in both countries. Two reports assessing CHIKV seroprevalence in various animal species were identified, although all detected antibodies were found to be cross-reactive with SINV (S2 Table) [41]. One report of CHIKV infection rates in *A. aegypti* mosquitoes was identified in our search, in which 26% CHIKV prevalence was detected during a CHIKV outbreak in Yemen (S3 Table)[122]. No human incidence reports were identified.

Table 1.2 Summary of human prevalence studies for chikungunya virus in the Middle East and North Africa (n=24).

Table 1.2

Country, Ref.	Year(s) of study*	City or Governorate	Setting; population (age range, years)	Sampling	Assay type†	Assay make	Sample size	Prevalence	Additional testing & Comments
Djibouti (n=4)									
Salah [46]	1987	Djibouti City	Military; healthy soldiers	Conv.	IIFT	In-house	50	**0%**	
		Randa	Rural community; general pop.	Conv.	IIFT	In-house	69	**0%**	
		Djibouti City	Hospital; AFI patients	Conv.	IIFT	In-house	41	**2.4%****	The single IIFT+ subject was a native of Ethoipia and was cross-reactive with SINV
Andayi [48]	2010-11	Djibouti City	Household survey; general pop.(<1-100)	SRS	ELISA	In-house	914	**2.6%**	95.9% (23/24) ELISA+ were NT+
Egypt (n=2)									
Darwish [123]	1974*	Multiple	n/s; general pop. (<1-70)	n/s	HI	In-house	231	**0%**	
Darwish [124]	1985	Cairo	Hospital; AFI patients (>10)	Conv.	HI	In-house	55	**5.5%**	0% (0/55) were convalescent +
Iran (n=2)									
Saidi [52]	1970	Multiple	n/s	n/s	HI	In-house	394	**22.1%****	
Saidi [53]	1970-71	Caspian region	Community; children (1-6)	Conv.	HI	In-house	100	**0%**	
Kuwait (n=2)									
Ibrahim [120]	1966-68	Multiple	Multiple; blood donors, non-AFI patients, children (1-60)	Conv.	HI	In-house	627	**1.4%****	0.3% (2/627) with CHIKV-specific without SINV or SFV cross-reaction
Al-Nakib [56]	1979-82	Jabriya	Hospital; non-AFI patients (0-60+)	SRS	HI	In-house	502	**0.4%****	possible cross-reaction with SINV
Pakistan (n=1)									
Darwish [41]	1983*	Karachi	Hospital; patients	Conv.	CF	In-house	43	**2.3%****	possible cross-reaction with SINV
Somalia (n=1)									
Botros [98]	1987	Hargeysa	Refugee camp; AFI patients	Conv.	HI	In-house	28	**0.0%**	0% (0/10) convalescent samples tested were HI+
Sudan (n=8)									
Salim [121]	1973*	Sennar	Community and clinical setting; general pop. and non-AFI patients (<1-40+)	Conv.	NT	In-house	62	**12.9%****	23% (11/48) were also NT+ for ONNV
Omer [36]	1976	Gezira State	Rural community; general pop. (5-40+)	Conv.	HI	In-house	109	**24.8%****	0.9% (1/109) were also HI+ to SINV; 8.2% (9/109) were NT+ for CHIKV
Woodruff [104]	1986	Juba	Hospital; patients with history of fever within past 6 months and AFI patients (1-85)	Conv.	HI	In-house	130	**23.1%**	no observed cross-reaction with SINV, SFxV
McCarthy [105]	1988	Khartoum	Clinical setting; non-AFI patients	CC	ELISA	In-house	100	**11%**	1/100 (1%) were IgM+
		Khartoum	Clinical setting; AFI patients (1-89)	CC	ELISA	In-house	196	**10%**	1/200 (0.5%) were IgM+
Watts [19]	1989	Northern state	Clinical setting; AFI patients (11-70)	Conv.	ELISA	In-house	185	**12.0%**	
Farnon [26]	2005	Kortalla	Community; general pop. (0-44+)	SSCS	ELISA	In-house	84	**43%**	1% (1/84) was CHIKV IgM+
Gould [20]	2005	South Kordofan	Clinical setting; suspected YF patients (n=3), severe illness (n=8), AFI patients (n=7), healthy (n=16)	Conv.	ELISA IgM	In-house	34	**23.5%**	
Tunisia (n=1)									
Nabli [125]	1970*	Multiple	n/s; children	Conv.	HI	In-house	100	**0%****	0.2% (3/1406) were HI+ for SINV
Yemen (n=3)									
Malik [116]	2010-11	Al-Hudaydah	Clinical setting; AFI patients (0-45+)	Conv.	ELISA IgM	In-house	136	**28%**	40% (54/136) were PCR+

Table 1.2 Continued

									22% (30/136) were cell culture +
Madani [117]	2010	Hadramout	Clinical settings; suspected viral hemorrhagic fever (3-75)	Conv.	PCR	In-house	222	0%	
Rezza [118]	2012	Al Hudaydah	Hospitals; AFI patients with 'dengue-like' illness (1-60)	Conv	ELISA IgM	NovaLisa	400	9.8%	2.8% (11/400) were PCR+ 9.4% (33/351 negative IgM/PCR) were IgG+

* Indicates year of publication when year(s) of data collection not available in report.
† All serologic assays were IgG unless otherwise stated.
**Indicates documented occurrence or suspicion of false-positives due to cross-reactions with other same family viruses or low serologic titers.
Abbreviations: AFI, acute febrile illness patients; CF, complement fixation; Conv, convenience; ELISA, enzyme-linked immunosorbent assay; HI, hemagglutinin inhi
indirect immunofluorescence test;
n/s, not specified; ONNV, O'nyong-nyong virus; pop., population; PCR, polymerase chain reaction; SFV, Semliki Forest virus; SFxV, Sandfly Fever virus; SIN\
virus; SRS, simple random sampling;
SSCS, single stage cluster sampling; VNT, viral neutralization test
Assay Abbreviation: NovaLisa (Dietzenbach, Germany)

Yellow Fever Overview. A total of 17 seroprevalence studies conducted from 1952-2011 were identified from eligible reports for YFV (Table 1.3). The geographic distribution of all YFV prevalence studies are illustrated in Figure 1.2. Anti-YFV antibodies were reported from populations in Djibouti, Lebanon, Somalia, Sudan, and Turkey. Neutralization testing or other secondary/confirmatory testing was performed in 7 (41%) studies, supporting the presence of anti-YFV antibodies in populations in Djibouti and Sudan but not in Turkey. Two reports containing seroprevalence measures for various animal species in Sudan were also identified (S2 Table), and there were no reports of YFV human incidence or vector infection rates identified from the region.

Table 1.3 Summary of human prevalence studies for yellow fever virus in the Middle East and North Africa

Table 1.3

Country, Ref.	Year(s) of study*	City or Governorate	Setting; population (age range, years)	Sampling	Assay type†	Assay make	Sample size	Prevalence	Additional testing & Comments
Djibouti (n=5)									
Salah [46]	1987	Djibouti City	Military; healthy soldiers (n/s)	Conv.	IIFT	In-house	50	0%	
		Randa	Rural community; general pop. (n/s)	Conv.	IIFT	In-house	69	0%	
		Djibouti City	Hospital; AFI patients (n/s)	Conv.	IIFT	In-house	41	0%	
Rodier [37]	1991	Djibouti City	Clinical setting; AFI patients (1-55) (YF vaccine recipients were included)	Conv.	ELISA IgM	In-house	91	10.9%	18.5% (5/27) ELISA+ samples were VNT+
Andayi [48]	2010-11	Djibouti City	Household survey; general pop.(<1-100)	SRS	ELISA	In-house	903	1.6%	90.9% (10/11) ELISA+ samples were VNT+
Egypt (n=2)									
Smithburn [126]	1954*	Upper Egypt	Rural communities and clinical setting; general pop. ('children and adults')	Conv.	VNT	In-house	331	0%	
Mohammed [50]	1968	Alexandria	Hospital; AFI patients (3-13)	Conv.	HI, CF	In-house	120	0%**	14.5% (7/48) were + by convalescent sera
Lebanon (n=1)									
Garabedian [58]	1962-65	Multiple	Community; general pop. (0-41+)	SRS	HI	In-house	115	61.7%**	
Somalia (n=2)									
Henderson [127]	1966	Giohar	Community; general pop. (<6-55+) (none with history of YF vaccination)	Conv.	HI	In-house	242	53.3%	
					CF	In-house	88	0%	
					MPT	In-house	71	8.4%	
Botros [98]	1987	Hargeysa	Refugee camp; AFI patients (n/s)	Conv.	IFA	In-house	28	0%	0% (0/10) convalescent sera were IFA+
Sudan (n=5)									
Taylor [128]	1952-54	Multiple	Community; general pop. ('all ages')	n/s	VNT	In-house	666	14.5%	
Salim [121]	1973	Sennar	Community and clinic; general pop., non-AFI patients (<1-40+) (none with history of YF vaccination)	Conv.	HI	In-house	62	95.1%	6.4% (4/62) were VNT+
Woodruff [104]	1986	Juba	Hospital; Patients with a history of fever within 6 months, AFI patients (1-85)	Conv.	HI	In-house	130	0.8%	
Watts [19]	1989	Northern Province	Clinical setting; AFI patients (11-70)	Conv.	ELISA	In-house	185	39%	
Gould [20]	2005	South Kordofan	Clinical setting; suspected YF patients (n=3), severe illness (n=8), AFI patients (n=7), healthy subjects (n=16) (none with history of YF vaccination)	Conv.	ELISA IgM	In-house	34	17.6%**	17.6% had anti-YFV antibodies without cross-reaction to DENV, WNV.
Turkey (n=1)									
Ergunay [113]	2010*	Ankara, Konya, Eskisehir, Zonguldak	Blood donor center; general pop. (n/s)	Conv.	ELISA	Euroimmun	1502	0.6%	0% (0/9) were mosaic IIFT+ (Euroimmun) or VNT+
Yemen (n=1)									
Madani [117]	2010	Hadramout	Clinical settings; suspected viral hemorrhagic fever (3-75)	Conv.	PCR	In-house	222	0%	

Table 1.3 Continued

* Indicates year of publication when year(s) of data collection not available in report.
† All serologic assays were IgG unless otherwise stated.
**Indicates documented occurrence or suspicion of false-positives due to cross-reactions with other same family viruses or low serologic titers.
Abbreviations: AFI, acute febrile illness patients; CF, complement fixation; Conv, convenience; ELISA, enzyme-linked immunosorbent assay; HI, hemagglutinin inhibition; IIFT, indirect immunofluorescence test; MPT, mouse-protection test; n/s, not specified; pop., population; PCR, polymerase chain reaction; SRS, simple random sampling; VNT, viral neutralization test
Assay Abbreviation: Euroimmun (Lubeck, Germany)

Overview of Outbreaks. Reported outbreaks of DENV, CHIKV, and YFV in the region were gathered through citations collected from the search databases (S4 Table) and mapped along with the geographic distribution of prevalence studies in Figure 1.2. For DENV, 81 outbreaks were reported from 9 countries in the region from 1941-2015, including sentinel reports of autochthonous transmission in Egypt (2010), and Yemen (1983). For CHIKV, 4 outbreaks were reported from Djibouti, Sudan, and Yemen, along with a sentinel report of autochthonous transmission in Saudi Arabia (2011). For YFV, 6 outbreak reports were identified, all recorded in Sudan from 1940-2013.

Pooled mean prevalence estimates for Dengue, Chikungunya, and Yellow Fever. The results of the pooled IgG seroprevalence estimates for both general and AFI populations are summarized in Table 1.4. The pooled IgG seroprevalence estimates among general populations in the MENA region were: DENV 8.6% (95% CI: 5.3-12.6%), CHIKV 4.3% (95% CI: 1.1-9.1%), and YFV 3.7% (95% CI: 0.2-10.4%). Among AFI populations, higher pooled prevalence estimates were estimated as follows: DENV 18.6% (95% CI: 7.4-33.3%), CHIKV 8.8% (95% CI: 4.9-13.6%), and YFV 3.3% (95% CI: 0.0-22.4%). The forest plots for each of these meta-analyses can be found in S7-S12 Figures. There was substantial evidence for heterogeneity in effect size in all meta-analyses, with p-value always <0.0001. Variation in effect size, rather than chance, accounted for the majority of the variation in all meta-analyses (I^2 >60%). The prediction intervals for each of these studies were broad, also highlighting substantial heterogeneity in effect size across studies.

Table 1.4 Pooled mean estimates for dengue, chikungunya, and yellow fever IgG seroprevalence stratified by general population and populations with undifferentiated acute febrile illness (AFI)

Table 1.4

	Studies	Samples	Prevalence	Effect size		Heterogeneity measures			
	Total N	Total N	Range (%)	Mean (%)	95% CI	Q (p-value)	τ^2	I² (confidence limits)	Prediction interval (%)
Dengue virus									
General population	47	16462	0.0-61.9	8.8	5.5-12.6	2735.0 (p<0.0001)	0.1722	98.3% (98.1-98.5)	0.0-44.5
AFI population	13	2332	0.0-87.5	18.6	7.4-33.3	692.31 (p<0.0001)	0.3619	98.3% (97.8-98.6)	0.0-82.9
Chikungunya virus									
General population	14	3385	0.0-42.8	4.3	1.1-9.1	348.13 (p<0.0001)	0.1166	96.3% (95-97.2)	0.0-32.3
AFI population	7	109	0.0-23.0	8.8	4.9-13.6	27.6 (p<0.0001)	0.0281	78.3% (55-89.5)	0.0-26.8
Yellow Fever									
General population	11	4071	0.0-61.7	3.7	0.2-10.4	581.14 (p<0.0001)	0.1805	98.3% (97.8-98.7)	0.0-42.3
AFI population	5	504	0.0-38.9	3.3	0.0-22.4	167.93 (p<0.0001)	0.4415	97.6% (96.2-98.5)	0.0-96.4

* τ^2 is the estimated between-study variance in the double arcsine transformed proportions.
The back-transformed τ^2 was not calculated as the methodology to do so is not currently available.

A multivariable meta-regression for DENV general and AFI population prevalence measures identified assay type as the only statistically significant prevalence-modifiable factor when adjusted for country, year of study, study precision, and sampling method. Hemagluttinin inhibition and immunoflouresence antibody tests were significantly more likely to yield lower prevalence values than ELISA or complement fixation tests. There was not sufficient statistical power to assess the effect size contribution of viral neutralization testing since there was only one prevalence measure using this assay type. Given the smaller number of prevalence studies for CHIKV and YFV general and AFI populations, we did not conduct meta-regressions to explore between-study heterogeneity in the pooled mean prevalence estimates for these viruses.

Quality Assessment. A summary of the precision and risk of bias assessment for human seroprevalence studies for each of the study viruses is located in S5 Table. The quality assessment for each study can be found in S6-S11 Tables. In brief, most studies (62-76%) contained high precision as defined by a sample size of ≥100 participants. Approximately 80% of studies in all categories utilized convenience samples and over 90% did not report response rates, entailing *high* and *unclear ROB*, respectively.

DISCUSSION

Our study offers the first systematic assessment of the current knowledge on the epidemiology of dengue, chikungunya, and yellow fever viruses in the MENA region from the standpoint of published prevalence and incidence data

and outbreak reports from the region. The results of our study indicate two apparent risk zones in the MENA harboring substantial circulation of *Aedes*-borne viruses: 1) Pakistan and 2) the Red Sea subregion. No human seroprevalence data was identified across broad subregions of the MENA, however, including some *Aedes* endemic areas. Moreover, our study identified a paucity of reports estimating human incidence and vector infection rates in the region. These limitations, along with the high study diversity and statistical heterogeneity identified in the meta-analyses of seroprevalence studies, simultaneously challenge efforts to synthesize and compare the inter- and intra-country epidemiology of the study viruses in the region while also revealing priorities for future research.

Dengue, Chikungunya, and Yellow Fever in the MENA region

Dengue virus. DENV is a globally distributed flavivirus that has a historic presence in the MENA region, with outbreaks of DENV and DENV-like disease reported across much of the Eastern Mediterranean region in the 19th and early 20th century [58, 129]. Our study demonstrates the presence of DENV focusing in two areas of the MENA: Pakistan and the Red Sea region. Pakistan demonstrated the highest number of reported prevalence studies and the broadest study coverage among MENA countries, suggesting substantial infection pressures in the country. Multiple studies reported >20% prevalence in both general population and those with AFI in the country [107, 130-133], and unlike other MENA countries, DENV serotypes 1-4 are all known to circulate in Pakistan [83]. Moreover, Pakistan reported the largest number of

confirmed cases among the DENV outbreaks in the MENA, with 21,580 cases reported during the 2011 outbreak of DENV-2 [80, 134] (S4 Table).

In the Red Sea region, our study identified multiple general population IgG seroprevalence measures and/or IgG seroprevalence measures among populations with AFI exceeding 20% in Djibouti, Saudi Arabia, Somalia, Sudan, and Yemen within the past decade (Table 1.1) along with multiple confirmed outbreaks of DENV serotypes 1-3 since the 1980s (S4 Table). Notably, the presence of DENV-4 has not yet been identified in this subregion to our knowledge. Although most reported outbreaks localize along the Red Sea coastline in these countries, seroprevalence studies suggest a broader distribution of DENV infections that are likely undetected throughout the subregion (Figure 1.2). This historic underdetection is also illustrated by the report of a traveler diagnosed with dengue after returning from Yemen in 1983 [135], followed by the first detected outbreaks of DENV in Yemen and Saudi Arabia 11 years later in 1994 [136-138]. In Egypt, our search identified no published prevalence studies or outbreaks after 1969 until a DEN outbreak was reported in November 2015 [11]. However, DENV transmission was suggested years beforehand by a report of two travelers diagnosed with DEN after returning from southern Egypt in 2011 [139] and the identification of *A. aegypti* in southern Egypt that same year [140]. Hence, whether the increasingly reported outbreaks from the region represent increasing incidence, increasing detection, or both, is not clearly defined amidst the high heterogeneity in study coverage and reporting across time and space in the MENA region (further described below).

Chikungunya virus. Chikungunya virus has traced the epidemiology of DENV in recent years to also establish itself as a global pathogen [130]. The past decade, CHIKV was implicated in an Indian Ocean outbreak with over a million cases, novel autochthonous transmission in Mediterranean Europe in 2007, and in 2013, novel transmission throughout the Caribbean islands and Latin America [141-146]. In our study, serologic evidence of CHIKV transmission in the MENA region has been published since the 1970s [9, 36, 41, 52, 116]. Like DENV, published seroprevalence studies and outbreaks appear to localize to the Red Sea region and Pakistan (Figure 1.2), with IgG seroprevalence in general populations and those with AFI measured over 20% in both Sudan and Yemen and 2% in Djibouti and Pakistan (Table 1.2). However, unlike DENV, confirmation of CHIKV in the MENA region was not published until 2010 during an outbreak in Yemen with over 15,000 suspected cases [116, 147]. This, along with the overall paucity of CHIKV prevalence studies and outbreak reports identified in our study, suggests a potentially substantial underrecognition of CHIKV in the MENA.

Yellow Fever virus. In the MENA region, YFV outbreaks have remained limited to Sudan, which lies within the Yellow Fever Belt of Africa (and S4 Table). Sporadic epidemics have been recorded in the country since 1940, primarily centered in the Nuba Mountain region in the south of the country [10, 128, 148]. Yet despite the longstanding availability of an effective vaccine, outbreaks of yellow fever were recently detected in South Kordofan and Darfur in 2005 and 2012, respectively [10, 148]. Moreover, seroprevalence studies have indicated widespread seroprevalence throughout the country (Table 1,3), though some measures raise the possibility of cross-reactions with other

flaviviruses or detection of vaccine-derived antibodies [37, 48, 50, 58, 113, 127]. Still, risk of YFV introduction must be considered for countries outside of Somalia and Sudan, particularly in *A. aegypti* endemic areas of Djibouti, Yemen, and Saudi Arabia [21].

Study Diversity and Limitations. An important finding our study was the substantial clinical and methodological diversity encountered among prevalence studies and in the meta-analyses for each virus. Clinically, studies represented a diversity of human populations of different ages and demographics, in different years, and in different locations and transmission contexts. A high proportion of studies were conducted decades ago when less stringent study methodologies or reporting standards may have been more common. This was also reflected in the assays utilized, with multiple studies utilizing assays susceptible to serologic cross-reactions and few utilizing confirmatory testing such as VNT. The meta-regression for DENV studies identified assay type as a statistically significant prevalence modifying factor. However, caution must be taken in interpreting the significance of this finding given the number of potentially prevalence-modifying covariates not included in the analysis, such as participant demographics or study context. Methodologically, most studies utilized convenience samples without reporting response rates, entailing the risk of bias and uncertainty in the accuracy of reported measure (S5 Table).

Meta-analyses for general populations and populations with AFI also demonstrated high statistical heterogeneity in effect size in all subgroups with wide prediction intervals (26-96%), entailing high uncertainty for the effect

estimates generated. Such statistical heterogeneity was expected given the apparent clinical and methodological diversity and wide rage of reported prevalence values. Nevertheless, this heterogeneity represents a challenge to interpreting the body of prevalence data that currently exists in the MENA.

Risk Assessment and Research Priorities. Our study did not identify any evidence confirming autochthonous transmission of DENV, CHIKV, or YFV in any of the MENA countries West of Egypt and East of Saudi Arabia until Pakistan (Figure 1.2). However, the paucity of published epidemiologic data in these sub-regions does not preclude the possibility of unrecognized transmission in some areas or the risk of emergence in others. Indeed, modeling studies suggest ecologic niches for *Aedes* along the coastal Mediterranean Basin of North Africa [131, 132, 149], and the presence of *A. albopictus* has been recently reported in Algeria, Lebanon, Palestine, Syria, and Turkey [58, 150-154]. In contrast*, A. albopictus* has been identified along the Mediterranean coast of Europe for decades, and local transmission of CHIKV in Italy and France and DENV in France and Croatia has been identified since 2007 [155]. Near the Pakistan border, seroprevalence studies also suggest the possibility of DENV transmission in Iran [13, 52, 54] and Afghanistan [45], though confirmatory investigations in these regions have not been published to our knowledge [54]. Given the limited human and vector surveillance in these regions, the presence of *A. aegypti* or *A. albopictus,* or the possibility of unrecognized dengue transmission, cannot be ruled out [54].

Moreover, the MENA region exhibits a variety of ecologic and social factors that may promote the spread of *Aedes*-borne viruses. Increased urbanization

in the region [156] promotes population crowding that augments the risk of outbreaks and, in some areas, use of open water storage containers that promote *A. aegypti* breeding [38, 56, 96, 111, 122, 131, 132, 149]. Unusually heavy rainfall or flooding has been implicated as an inciting factor in multiple DENV outbreaks in Sudan, Djibouti, and Yemen [9, 48, 157, 158], phenomena which may grow increasingly unpredictable as climate change affects the region. Armed conflicts and economic turmoil, as that which has occurred in Yemen, Syria, and Iraq, may render previously stable communities vulnerable to vector-borne disease transmission while further disabling public health surveillance and outbreak response capacity [159]. Inter-regional migration pose risk for imported arboviruses as well, as millions of migrants travel from DENV and CHIKV endemic countries in Asia and North Africa to the Arabian Peninsula to work [111, 136, 147, 159-161] and millions more travel annually to Mecca, Saudi Arabia (a DENV endemic region) to attend the Umra and Hajj [136]. Intra-regionally, heavy human travel and trade in the Red Sea region likely drives arbovirus mixing and spread [116, 161], as evidenced by multiple DENV outbreaks occurring at Red Sea port cities in Djibouti [37, 46], Sudan [38] Yemen [116, 161], and Saudi Arabia [136]. Risk of contiguous spread of DENV from Yemen to Oman [162] or from Pakistan to Iran or Afghanistan [13] has been raised.

The results of our study emphasize the need for further research into the prevalence and distribution of *Aedes*-borne arboviruses in the MENA region. Our study was limited by its reliance on select databases of peer-reviewed literature with the exclusion of grey literature which may have provided additional data. In addition, including other *Aedes*-transmitted pathogens or

studies reporting *Aedes* distribution in the MENA may have provided further insights regarding the geographic distribution of the study viruses. Nevertheless, a number of research priorities emerge concerning these pathogens in the MENA.

First, broader seroepidemiologic survey coverage in the region is needed. Such studies are efficient means of characterizing infection pressures in populations lacking public health surveillance and diagnostic capacity, particularly when transmission status is uncertain. Multiplexed diagnostics are increasingly available for such purposes and are well-suited for simultaneously exploring the possible distribution of a number of other undercharacterized arboviruses in the region (e.g. Alkhumra, Crimean-Congo Hemorrhagic Fever, O'Nyong-nyong, Rift Valley Fever, Sandfly Fever virus complex, Usutu, West Nile viruses). Second, future seroprevalence studies include methods to minimize serologic cross-reactions whenever possible, particularly for the flavivirus serocomplexes [163]. Third, seroepidemiologic studies should incorporate uniformity in study design and enrollment criteria to minimize confounding, such as the standard case definitions or for studies of 'suspected' dengue infection put forth by the WHO [164]. Ideally this could include population-based sampling studies, which could provide baseline data from which to benchmark the impact of these pathogens in the region over the coming years. Fourth, future studies should incorporate vector surveillance and studies of infection rates, as our study identified a paucity of vector infection rates in the MENA. Such studies are important for understanding transmission dynamics that inform vector control strategies, predict future transmission activity, and project disease risk to humans [131, 149, 155].

Guidelines and standard tools for calculating vector infection rates are available [155, 165].

Our study provides a comprehensive systematic summary of the evidence supporting the epidemiology of dengue, chikungunya, and yellow fever in the MENA region. The available seroprevalence and outbreak data clearly suggests the Red Sea region and Pakistan are transmission zones for DENV and CHIKV in the MENA, while YFV remains confined to Sudan. However, high study diversity and statistical heterogeneity exists among studies and broad areas lack published data, particularly concerning incidence rates and vector infection rates in the MENA. These findings serve as a resource for future arbovirus research planning in the MENA by articulating epidemiologic knowledge gaps and the need for well-designed seroepidemiologic and vector studies. Such studies are essential to assess and mitigate the impact of arbovirus disease in the MENA region.

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APPENDIX 1

SUPPORTING INFORMATION

SUPPORTING INFORMATION

The Epidemiology of Dengue, Chikungunya, and Yellow Fever in the Middle East and North Africa: Systematic Review and Meta-Analysis

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Short title: Dengue, Chikungunya, and Yellow fever in the MENA Region

S1 Figure Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	5-6
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6-7
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	7-8, S3 Fig.
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7 and S2 Fig.
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	S2 Fig.
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7-8
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	8-9, Table 1-3, S4-S5 Figs.
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	8,9,14, S6 Fig.
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	9-10

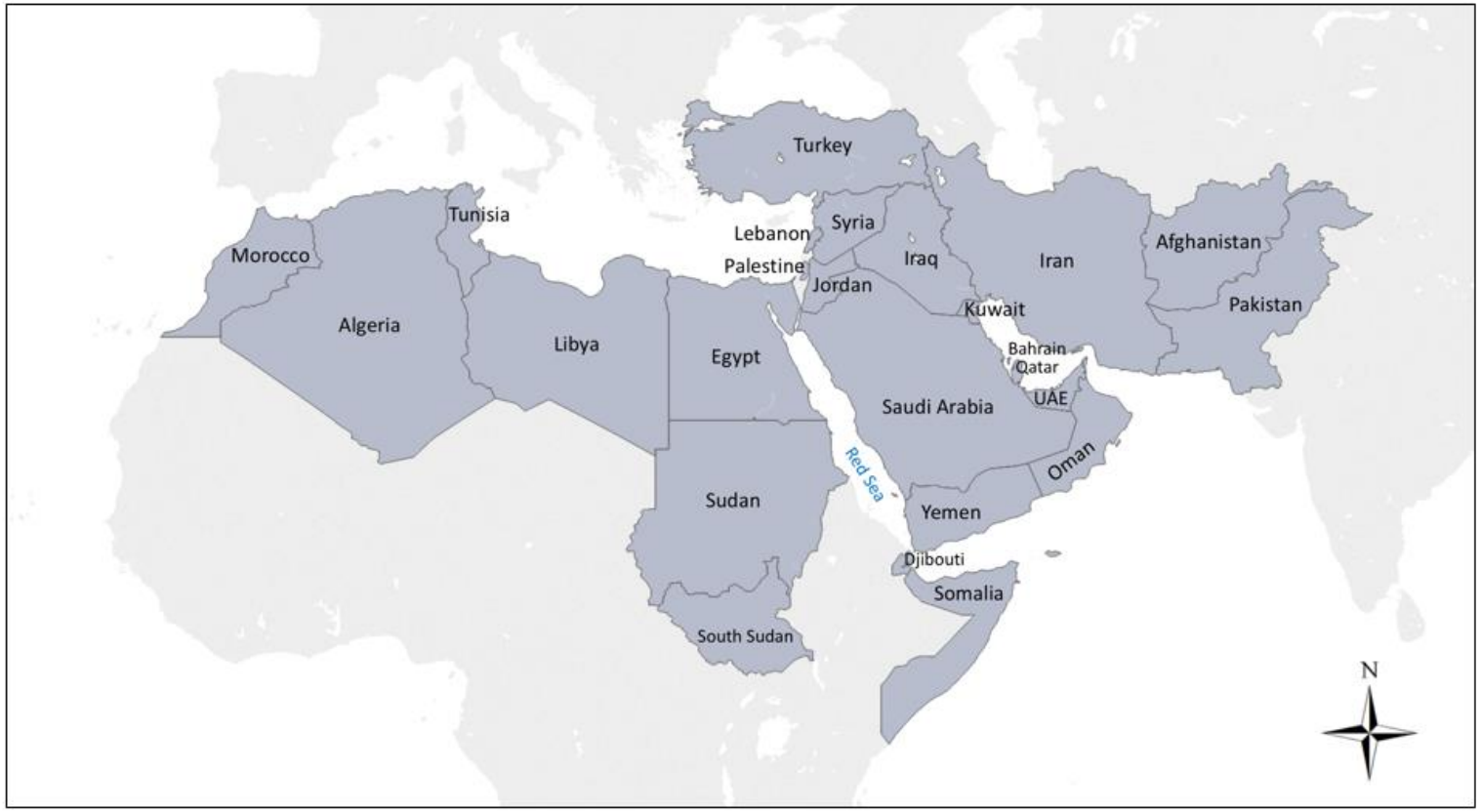
S2 Figure Data sources and search criteria used for the systematic review of dengue, chikungunya, and yellow fever virus prevalence and incidence in the Middle East and North Africa.

Dengue Search Criteria
PubMed (Last searched December 9, 2015)
("Dengue"[text] OR "Dengue"[mesh] OR "Dengue Virus"[mesh] OR “Aden fever”[text] or “bouquet fever”[text] or “breakbone fever”[text] or “dandy fever”[text] or “red fever”[text] or “solar fever”[text] or “sun fever”[text] OR Flavivir*[text] OR “Arbovirus, Group B”[Mesh]) **AND** ("Middle East"[Mesh] OR "Islam"[Mesh] OR "Arabs"[Mesh] OR "Arab World"[Mesh] OR "Africa, Northern"[Mesh] OR "Sudan"[Mesh] OR "Somalia"[Mesh] OR "Djibouti"[Mesh] OR "Pakistan"[Mesh] OR "Middle East"[Text] OR "Middle-East"[Text] OR "North Africa"[Text] OR "North-Africa"[Text] OR "EMRO"[Text] OR "Eastern Mediterranean"[Text] OR “Arab”[Text] OR “Arabs”[Text] OR “Arab World”[Text] OR "Islam"[Text] OR "Afghanistan"[Text] OR "Algeria"[Text] OR "Bahrain"[Text] OR "Djibouti"[Text] OR "Egypt"[Text] OR "Jordan"[Text] OR "Kuwait"[Text] OR "Lebanon"[Text] OR "Libya"[Text] OR "Iran"[Text] OR "Iraq"[Text] OR "Morocco"[Text] OR "Oman"[Text] OR "Pakistan"[Text] OR "Qatar"[Text] OR "Saudi Arabia"[Text] OR "Somalia"[Text] OR "Sudan"[Text] OR "Syria"[Text] OR "Tunisia"[Text] OR “Turkey”[text] OR "United Arab Emirates"[Text] OR "Dubai"[Text] OR "Abu Dhabi"[Text] OR "Abu-Dhabi"[Text] OR “Sharjah”[Text] OR "West Bank"[Text] OR "Ghaza"[Text] OR "Palestine"[Text] OR"Yemen"[Text])
Embase (Embase 1974 to 2015 Week 49; Last searched December 9, 2015)
(exp Dengue/ OR Dengue.mp OR Aden Fever.mp OR Bouquet Fever.mp OR Breakbone Fever.mp OR Dandy Fever.mp OR Red Fever.mp OR Solar Fever.mp OR Sun Fever.mp OR Flavivir*.mp OR Arbovirus, Group B.mp.) **AND** (exp Middle East/ or exp North Africa/ or exp Arab/ or exp Afghanistan/ or exp Djibouti/ or exp Pakistan/ or exp Somalia/ or exp Sudan/ or Middle East.mp. or North Africa.mp. or EMRO.mp. or Eastern Mediterranean.mp. or Arab.mp. or Arabs.mp. or Arab World.mp. or Islam.mp. or Afghanistan.mp. or Algeria.mp. or Bahrain.mp. or Djibouti.mp. or Egypt.mp. or Jordan.mp. or Kuwait.mp. or Lebanon.mp. or Libya.mp. or Iran.mp. or Iraq.mp. or Morocco.mp. or Oman.mp. or Pakistan.mp. or Qatar.mp. or Saudi Arabia.mp. or Somalia.mp. or Sudan.mp. or Syria.mp. or Tunisia.mp. or Turkey.mp or United Arab Emirates.mp. or Dubai.mp. or Abu Dhabi.mp. or Sharjah.mp. or West Bank.mp. or Ghaza.mp. or Palestine.mp. or Yemen.mp.)
Chikungunya Search Criteria
PubMed (Last searched December 9, 2015)
("Chikungunya"[text] OR "Chikungunya Virus"[mesh] OR “CHIK”[text] OR Alphavir*[text] OR “Arbovirus, Group A”[Mesh]) **AND** ("Middle East"[Mesh] OR "Islam"[Mesh] OR "Arabs"[Mesh] OR "Arab World"[Mesh] OR "Africa, Northern"[Mesh] OR "Sudan"[Mesh] OR "Somalia"[Mesh] OR "Djibouti"[Mesh] OR "Pakistan"[Mesh] OR "Middle East"[Text] OR "Middle-East"[Text] OR "North Africa"[Text] OR "North-Africa"[Text] OR "EMRO"[Text] OR "Eastern Mediterranean"[Text] OR “Arab”[Text] OR “Arabs”[Text] OR “Arab World”[Text] OR "Islam"[Text] OR "Afghanistan"[Text] OR "Algeria"[Text] OR "Bahrain"[Text] OR "Djibouti"[Text] OR "Egypt"[Text] OR "Jordan"[Text] OR "Kuwait"[Text] OR "Lebanon"[Text] OR "Libya"[Text] OR "Iran"[Text] OR "Iraq"[Text] OR "Morocco"[Text] OR "Oman"[Text] OR "Pakistan"[Text] OR "Qatar"[Text] OR "Saudi Arabia"[Text] OR "Somalia"[Text] OR "Sudan"[Text] OR "Syria"[Text] OR "Tunisia"[Text]

S2 Figure Continued

Embase (Embase 1974 to 2015 Week 49; Last searched December 9, 2015)
(exp Chikungunya/ OR exp Chikungunya virus/ OR Chikungunya.mp OR CHIK.mp OR Alphavir*.mp or group A arbovirus.mp) **AND** (exp Middle East/ or exp North Africa/ or exp Arab/ or exp Afghanistan/ or exp Djibouti/ or exp Pakistan/ or exp Somalia/ or exp Sudan/ or Middle East.mp. or North Africa.mp. or EMRO.mp. or Eastern Mediterranean.mp. or Arab.mp. or Arabs.mp. or Arab World.mp. or Islam.mp. or Afghanistan.mp. or Algeria.mp. or Bahrain.mp. or Djibouti.mp. or Egypt.mp. or Jordan.mp. or Kuwait.mp. or Lebanon.mp. or Libya.mp. or Iran.mp. or Iraq.mp. or Morocco.mp. or Oman.mp. or Pakistan.mp. or Qatar.mp. or Saudi Arabia.mp. or Somalia.mp. or Sudan.mp. or Syria.mp. or Tunisia.mp. or Turkey.mp or United Arab Emirates.mp. or Dubai.mp. or Abu Dhabi.mp. or Sharjah.mp. or West Bank.mp. or Ghaza.mp. or Palestine.mp. or Yemen.mp.)
Yellow Fever Search Criteria
PubMed (Last searched December 9, 2015)
("Yellow Fever"[text] OR “YF”[text] OR "Yellow Fever Virus"[Mesh] OR "Yellow Fever"[Mesh] OR Flavivir*[text] OR “Arbovirus, Group B”[Mesh]) **AND** ("Middle East"[Mesh] OR "Islam"[Mesh] OR "Arabs"[Mesh] OR "Arab World"[Mesh] OR "Africa, Northern"[Mesh] OR "Sudan"[Mesh] OR "Somalia"[Mesh] OR "Djibouti"[Mesh] OR "Pakistan"[Mesh] OR "Middle East"[Text] OR "Middle-East"[Text] OR "North Africa"[Text] OR "North-Africa"[Text] OR "EMRO"[Text] OR "Eastern Mediterranean"[Text] OR “Arab”[Text] OR “Arabs”[Text] OR “Arab World”[Text] OR "Islam"[Text] OR "Afghanistan"[Text] OR "Algeria"[Text] OR "Bahrain"[Text] OR "Djibouti"[Text] OR "Egypt"[Text] OR "Jordan"[Text] OR "Kuwait"[Text] OR "Lebanon"[Text] OR "Libya"[Text] OR "Iran"[Text] OR "Iraq"[Text] OR "Morocco"[Text] OR "Oman"[Text] OR "Pakistan"[Text] OR "Qatar"[Text] OR "Saudi Arabia"[Text] OR "Somalia"[Text] OR "Sudan"[Text] OR "Syria"[Text] OR "Tunisia"[Text] OR “Turkey”[text] OR "United Arab Emirates"[Text] OR "Dubai"[Text] OR "Abu Dhabi"[Text] OR "Abu-Dhabi"[Text] OR “Sharjah”[Text] OR "West Bank"[Text] OR "Ghaza"[Text] OR "Palestine"[Text] OR"Yemen"[Text])
Embase (Embase 1974 to 2015 Week 49; Last searched December 9, 2015)
(exp Chikungunya/ OR exp Chikungunya virus/ OR Chikungunya.mp OR CHIK.mp OR Alphavir*.mp or group A arbovirus.mp) **AND** (exp Middle East/ or exp North Africa/ or exp Arab/ or exp Afghanistan/ or exp Djibouti/ or exp Pakistan/ or exp Somalia/ or exp Sudan/ or Middle East.mp. or North Africa.mp. or EMRO.mp. or Eastern Mediterranean.mp. or Arab.mp. or Arabs.mp. or Arab World.mp. or Islam.mp. or Afghanistan.mp. or Algeria.mp. or Bahrain.mp. or Djibouti.mp. or Egypt.mp. or Jordan.mp. or Kuwait.mp. or Lebanon.mp. or Libya.mp. or Iran.mp. or Iraq.mp. or Morocco.mp. or Oman.mp. or Pakistan.mp. or Qatar.mp. or Saudi Arabia.mp. or Somalia.mp. or Sudan.mp. or Syria.mp. or Tunisia.mp. or Turkey.mp or United Arab Emirates.mp. or Dubai.mp. or Abu Dhabi.mp. or Sharjah.mp. or West Bank.mp. or Ghaza.mp. or Palestine.mp. or Yemen.mp.)
Regional Databases
Index Medicus for the Eastern Mediterranean Region and African Index Medicus (Last searched December 9, 2015)
separate searches were conducted using the terms ‘dengue’, ‘chikungunya’, and ‘yellow fever’ OR “Turkey”[text] OR "United Arab Emirates"[Text] OR "Dubai"[Text] OR "Abu Dhabi"[Text] OR "Abu-Dhabi"[Text] OR “Sharjah”[Text] OR "West Bank"[Text] OR "Ghaza"[Text] OR "Palestine"[Text] OR "Yemen"[Text])

S3 Figure Map of the Middle East and North Africa (MENA) region. The definition adopted in the review includes the following 23 countries colored in grey: Afghanistan, Algeria, Bahrain, Djibouti, Egypt, Iran, Iraq, Jordan, Kuwait, Lebanon, Libya, Morocco, Oman, Pakistan, Palestine, Qatar, Saudi Arabia, Somalia, Sudan (including the newly established Republic of South Sudan), Syria, Tunisia, Turkey, United Arab Emirates (UAE), and Yemen.



S4 Figure Lists of variables extracted from relevant reports for dengue (DENV), chikungunya (CHIKV), and yellow fever (YFV) virus incidence and/or prevalence studies.

Extraction variable	Description
Author	
Full citation	
Country of survey	
Year(s) of data collection	*year of publication when data collection period not reported
Duration of data collection	for incidence studies only
City or Governorate	
Study setting	
Study population	including population age range in years, when available
Sampling method	
Assay type	complement fixation (CF) test, enzyme-linked immunosorbent assay (ELISA) IgG and IgM, immunofluorescence antibody assay (IFA), hemagluttinin inhibition (HI), NS1 antigen test, viral neutralization test (VNT), polymerase chain reaction (PCR), cell culture
Assay make	in-house or commercial, and name of commercial assay when available
Target protein	DENV only, whole virus or envelope protein
Assay serotype	DENV only, serotype tested and/or detected by assay
Sample size	
Prevalence	
Incidence	
Response rate	for risk of bias assessment (see ROB tables)
Additional testing and comments	includes secondary assays used for confirmatory testing, IgM or PCR prevalence, and comments regarding assay cross-reactivity in the study or serotypes detected by assay
animal species	animal prevalence studies only
mosquito species	vector infection rate studies only
infection rate	vector infection rate studies only

S5 Figure Classification of populations identified through the systematic review:

1. General population: these include seroprevalences studies among populations of individuals not suspected suspicion to have acute arbovirus infection at the time of the study. These populations include household members, blood donors, military personnel, students, and hospitalized patients and outpatients receiving care for other illnesses.

2. Acute febrile illness (AFI): these include studies of undifferentiated febrile illness for which dengue, chikungunya, or yellow fever infection cannot be discerned on clinical grounds alone. In these studies, IgG prevalence measures drawn during the acute phase of illness are more likely to indicate previous infection with that pathogen rather than acute infection.

3. Suspected dengue: these include cases in which dengue fever is suspected either by WHO-defined clinical criteria for probable dengue infection or when "suspected dengue" is stated as an inclusion criterion but is not further explained in the report.

S6 Figure Description of quality assessment criteria and studies' risk of bias (ROB) appraisal.

Description of quality assessment criteria and study risk of bias (ROB) appraisal for dengue (DENV), chikungunya (CHIKV), and yellow fever (YFV) virus human prevalence studies.

The quality of CHIKV, DENV, and YFV human prevalence measures identified through our review was determined by assessing:

1. The risk of bias (ROB) based on two quality domains
    a. The rigor of the sampling methodology
    b. The response rate

2. The precision of the reported measures
    Studies were considered to have high precision if the number of individuals tested was ≥ 100. The 95% CI is 0.7-9.2% for a sample size of 100 with a prevalence of 5%, which is a reasonable precision.

Study ROB appraisal

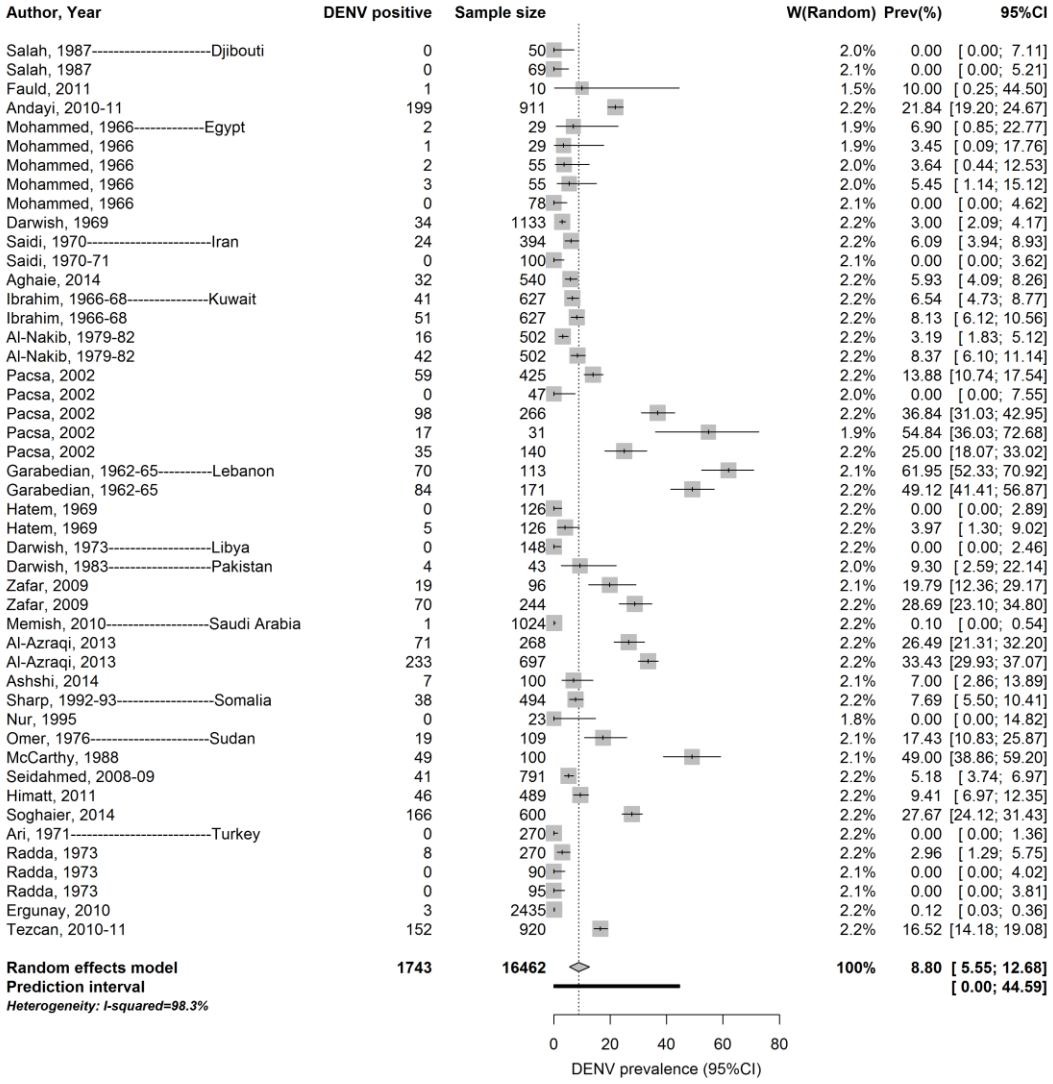
1. Low ROB
    a. Probability-based sampling
    b. Response rate ≥ 80%

2. High ROB
    a. Non-probability-based sampling
    b. Response rate < 80%

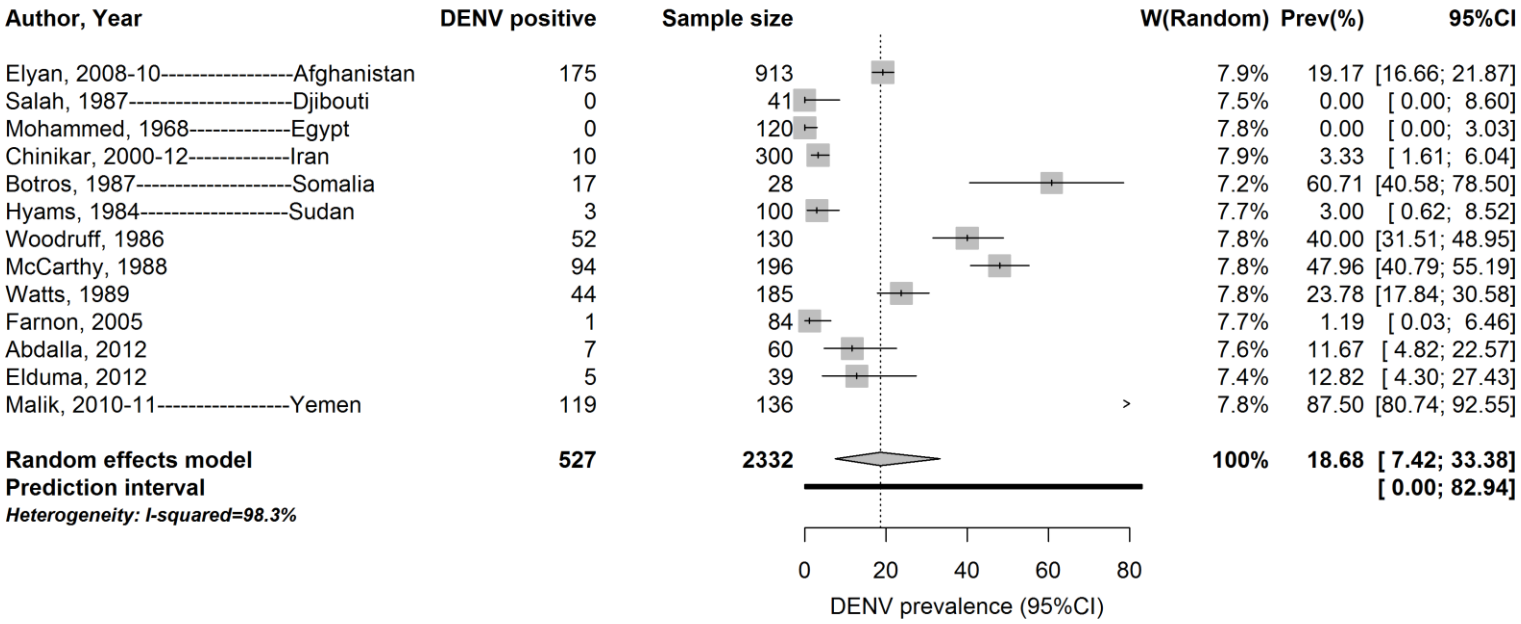
3. Unclear ROB
    Studies with missing information for any of the domains were classified as having *unclear* ROB for that specific domain

Note: 1) Prevalence measures among individuals presenting voluntarily to facilities where routine blood screening is conducted (i.e. blood donation center) were considered to have low ROB for the response rate domain.

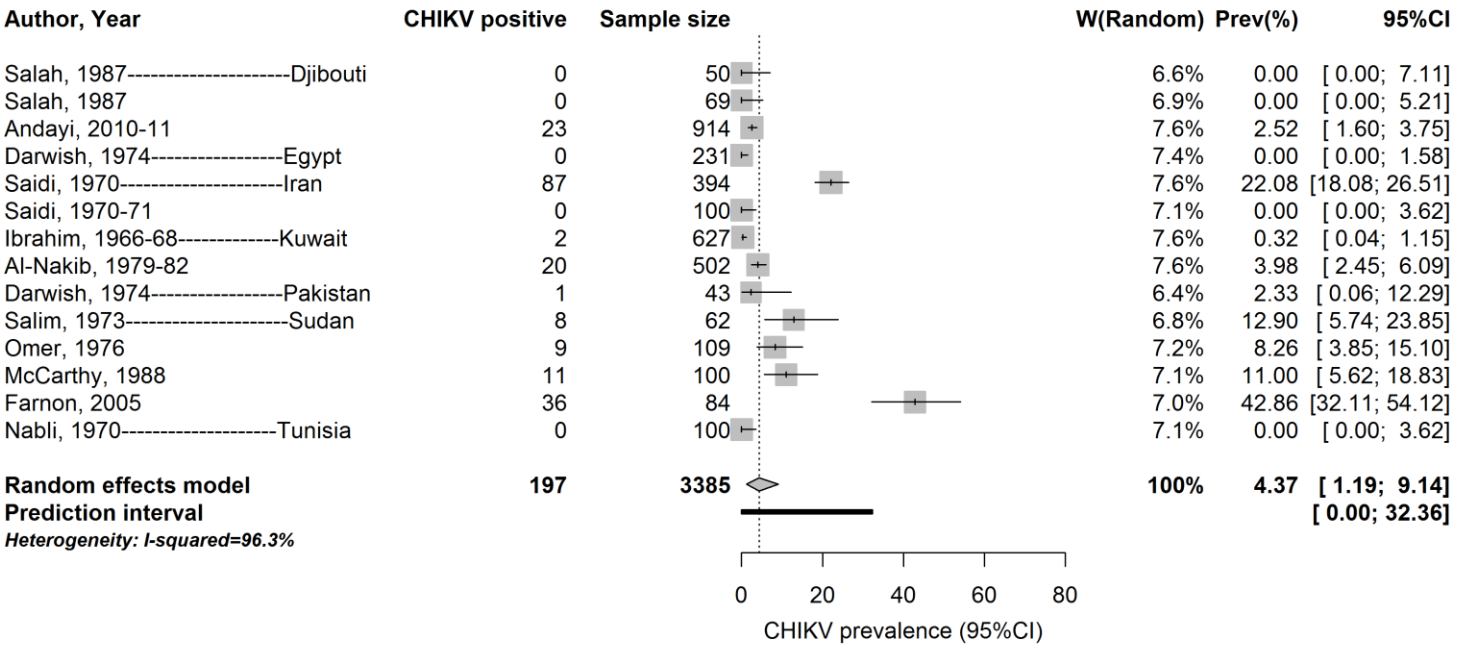
S7 Figure Forest plot presenting the results of the meta-analysis of dengue virus prevalence measures among general populations in the Middle East and North Africa.



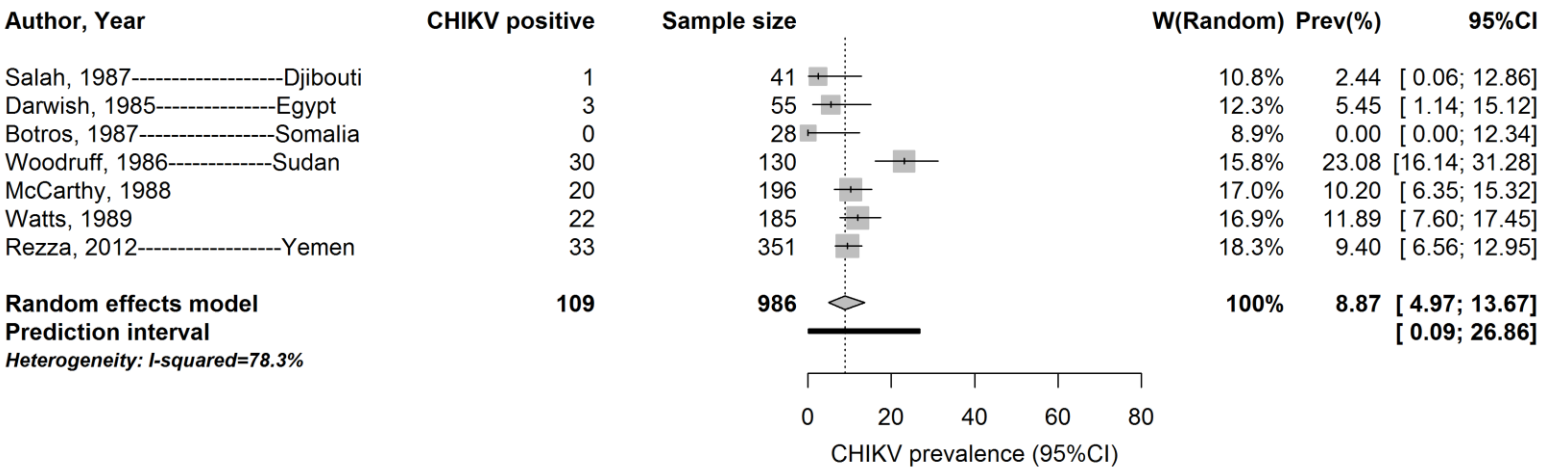
S8 Figure Forest plot presenting the results of the meta-analysis of dengue virus prevalence measures among acute febrile illness populations in the Middle East and North Africa.



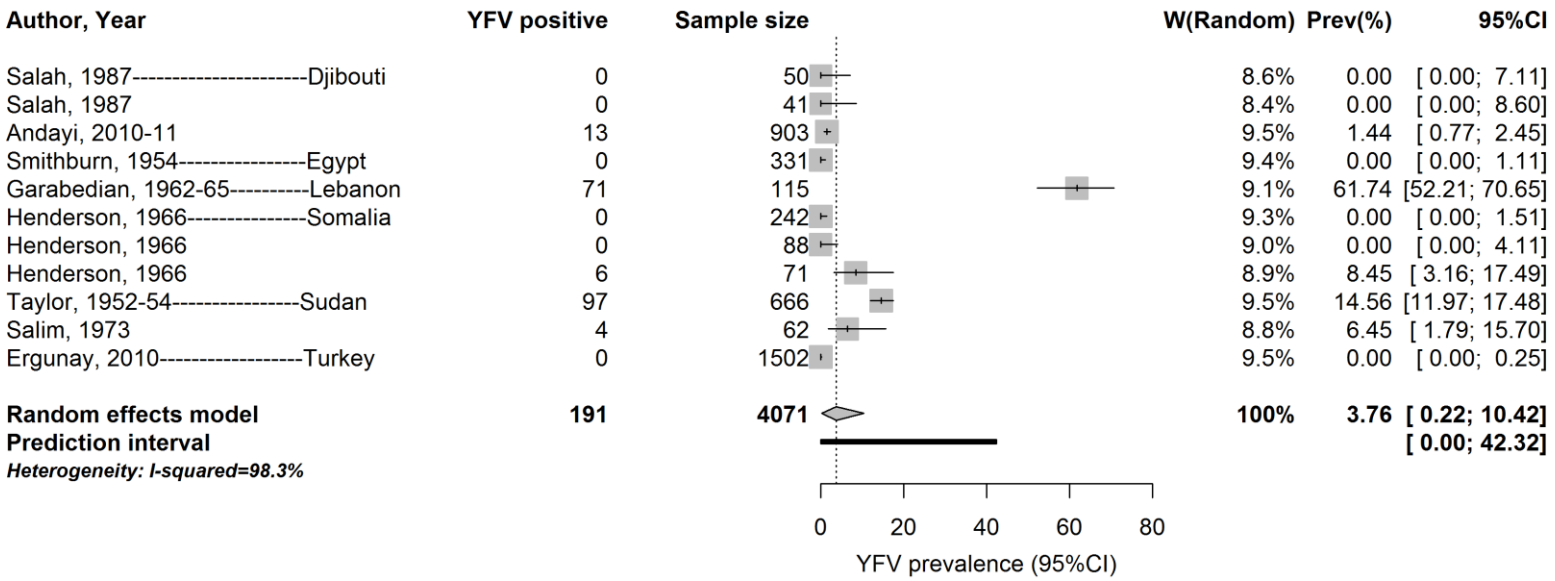
S9 Figure Forest plot presenting the results of the meta-analysis of chikungunya virus prevalence measures among general populations in the Middle East and North Africa.



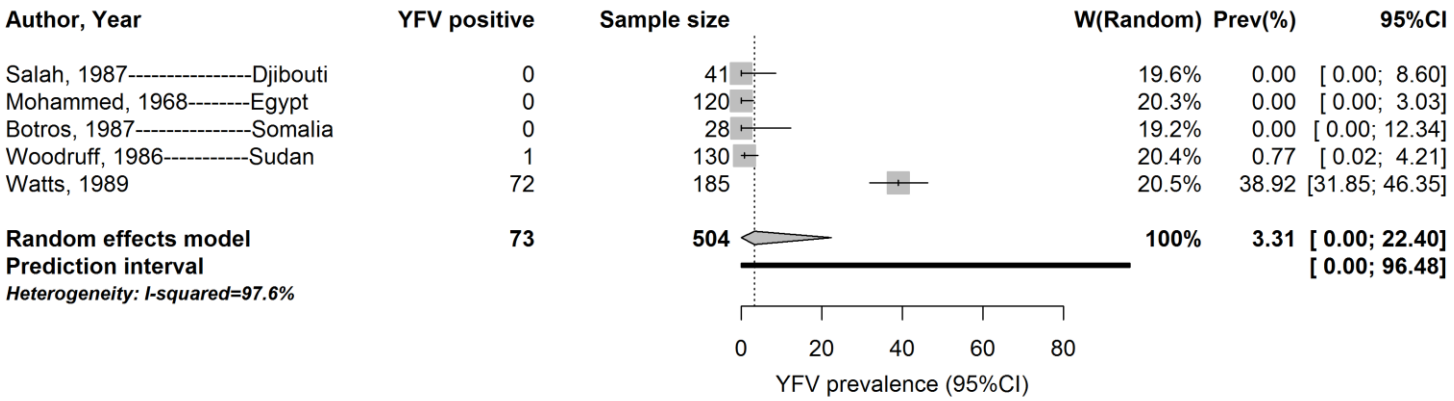
S10 Figure Forest plot presenting the results of the meta-analysis of chikungunya virus prevalence measures among acute febrile illness populations in the Middle East and North Africa.



S11 Figure Forest plot presenting the results of the meta-analysis of yellow fever virus prevalence measures among general populations in the Middle East and North Africa.



S12 Figure Forest plot presenting the results of the meta-analysis of yellow fever virus prevalence measures among acute febrile illness populations in the Middle East and North Africa.



S1 Table. Summary of human incidence studies for dengue virus in the Middle East and North Africa (n=3).

Country, Ref.	Year(s) of study	Duration of follow-up	City or governorate	Setting; population (age range, years)	Study design	Sampling	Assay type	Assay make*	Assay Target	Serotype tested	Sample size	Incidence
Pakistan (n=1)												
Siddiqui [1]	1999-2001	1999-2001	Karachi	Urban slum; children <16 years of age with undifferentiated febrile illness	CS	Active surveillance	ELISA IgM	Diag. Auto.	wv	1-4	1,248	**18.5/10,000**
Sudan (n=2)												
Seidahmed [2]	2008-09	12 months	Port Sudan City	Urban community; general pop. living in houses where DENV-carrying mosquitoes were present (<1-80)	Pros. coh	RSS	ELISA IgM	PanBio	Env	1-4	791	**35/10,000**
Seidahmed [3]	2010	17 weeks	Port Sudan	Urban community; general pop.	CS	Conv.	ELISA IgM, NS1, PCR	n/s	n/s	n/s	3,765‡	**94/10,000**

‡ reported cases

Abbreviations: CS, cross-sectional; ELISA, enzyme-linked immunosorbent assay; Env, envelope; PCR, polymerase chain reaction; Pros. coh, prospective cohort; RSS, random stratified sampling

Assay Abbreviation: *Diag. Auto. (*Diagnostic Automation, CA, USA); *PanBio* (Brisbane, Australia)

S2 Table. Summary of studies reporting dengue, chikungunya, and yellow fever virus seroprevalence among animals in the Middle East and North Africa.

Country, Ref.	Virus	Year(s) of data collection	City or Governorate	Setting	Assay type†	Assay make	Species	Sample size	Prevalence	Notes
Pakistan										
Darwish [4]	DENV	1983*	Karachi, Sind, Punjab	slaughterhouse, wilderness	CF	In-house	cattle	45	0%	DENV-1
					CF	In-house	sheep	46	10.9%	DENV-1
					CF	In-house	goat	48	0%	DENV-1
					CF	In-house	rodent	157	1.3%	DENV-1
					CF	In-house	buffalo	33	6.1%	DENV-1
	CHIKV				CF	In-house	goat	45	0%	
					CF	In-house	rodent	46	0%	
					CF	In-house	buffalo	48	0%	
					CF	In-house	rodent	157	2.5%**	Observed cross-reaction with SINV
Sudan										
Kirk [5]	YFV	1953*	Kau	community	CF / MPT	In-house	hens	6	0%	
			Western Kordofan		CF / HI	In-house	cattle	2	100%	59 of 62 samples (95%) were positive by HI
			White Nile		CF / MPT	In-house	cattle	4	0%	
			khartoum		CF / MPT	In-house	cattle	5	0%	
			Kau		CF / MPT	In-house	cattle	12	33.3%	
			Kau		CF / MPT	In-house	sheep	4	0%	
			Western Kordofan		CF / MPT	In-house	sheep	13	15.4%	
			Kau		CF / MPT	In-house	goat	3	0%	
			Kau		CF / MPT	In-house	pig	8	37.5%	
			Kau		CF / MPT	In-house	dog	3	66.7%	
			Sudan		CF / MPT	In-house	primate	19	10.5%	
Taylor [6]	YFV	1952	Multiple provinces in south of country	wilderness	NT	In-house	primate	101	39.6%	
Tunisia										
Chastel [7]	DENV	1976-77	Multiple	wilderness	HI	In-house	rodents	156	0.6%	DENV serotype not specified
Chastel [8]	DENV	1980	El Gharia, Ezzitoun	community	HI	In-house	small mammals	19	0%	DENV-2
			Iles Kerkhennah				small mammals	22	4.5%	DENV-2
			Zarzis, Tataouine, Medenine				small mammals	28	0%	DENV-2
			zaghouan, Moghrane, Ezzriba				small mammals	34	5.9%	DENV-2
Turkey										
Ari [9]	DENV	1971	W. Anatolia	community	HI	In-house	sheep	263	0.8%	potential for cross-reaction with WNV, YF, TBEV

* Indicates year of publication when year(s) of data collection not available in report.

† All serologic assays were IgG unless otherwise stated.

**Indicates documented occurrence or suspicion of false-positives due to cross-reactions with other same family viruses or low serologic titers.

Abbreviations: CF, complement fixation; CHIKV, chikungunya virus; DENV, dengue virus; HI, hemagglutinin inhibition; MPT, mouse-protection test; n/s, not specified; pop., population; SINV, sindbis virus; YFV, yellow fever virus

S3 Table. Summary of vector infection rate studies for dengue and chikungunya viruses in the Middle East and North Africa.

Author, Ref.	Virus	Year(s) of data collection	City or governorate	Setting	Mosquito species	Assay type	Sample size	Infection rate	Comments
Pakistan									
Jahan [10]	DENV	2011	Lahore	Urban areas	*A aegypti*	Ag-capture ELISA	114 pools (n=570 mosquitoes)	**27.2%**	
					A albopictus	Ag-capture ELISA	4 pools (n=20 mosquitoes)	**25%**	
Yemen									
Zayed [11]	DENV	2010-11	Al Hodayda	houses of CHIK cases at Eritrean refugee camp	*A aegypti*	RT-PCR	11 pools (n=30 mosquitoes)	**0%**	17 *Culex spp.* mosquitoes were negative for DENV RNA.
	CHIKV	2010-11	Al Hodayda	houses of CHIK cases at Eritrean refugee camp	*A aegypti*	RT-PCR	11 pools (n=30 mosquitoes)	**26.6%**	17 *Culex spp.* mosquitoes were negative for CHIKV RNA.

CHIKV, chikungunya virus; DENV, dengue virus; RT-PCR, reverse transcription-polymerase chain reaction

S4 Table. Summary of reported outbreaks and sentinel cases for dengue, yellow fever, and chikungunya virus in the Middle East and North Africa.

Country, Year	City or Governorate	Description	Ref.
Dengue virus			
Djibouti			
1991-92	Djibouti City	12,000 estimated cases; DEN-2	[13, 14]
2010-11	Djibouti City	4 cases in foreign military personnel; DEN-1,2,3	[15]
Egypt			
1799	Cairo, Alexandria	unconfirmed outbreak	[16]
1871	Port Said	unconfirmed outbreak	[16]
1880	Cairo	unconfirmed outbreak	[16]
1889	Nile Delta	unconfirmed outbreak	[16]
1906	Port Said, Suez Canal	unconfirmed outbreak	[17]
1927	Countrywide	unconfirmed outbreak	[17-19]
1937	n/a	n/a	[16]
2010	Port Ghalib	2 cases in travelers returning from Egypt	[16]
2015	Assiut	253 cases; DEN-1	[20]
Iraq			
1944	n/s	unconfirmed outbreak based on clinical diagnosis	[21, 22]
Lebanon			
1945-46	Beirut	>100,000 suspected cases	[23]
Pakistan			
1994-1995	Karachi	first confirmed outbreak of DENV in Pakistan; 145 reported cases; DEN-1,2	[25-30]
2003	n/a	DEN-1,2	[30, 31]
2005-06	Karachi	3,640 cases; DEN-3	[27, 28, 30, 32]
2006	Multiple	1,931 confirmed cases; DEN-2,3	[30, 33]
2007	Multiple	1,226 confirmed cases; DEN-2,3	[30, 33]
2008	Multiple	2,469 confirmed cases; DEN-2,3,4	[33, 34]
2009	Lahore, multiple	1,085 confirmed cases; DEN-2,3,4	[33]
2010	Multiple	11,024 confirmed cases	[33] [35]
2011	Punjab, multiple	21,580 confirmed cases; DEN-2	[33, 36]
2013	Khyber Pakhtunkhwa	9,024 reported cases	[37, 38]
	Sindh	>6,000 reported cases	[37]
2014	Lahore	1,799 reported cases	[39]
Saudi Arabia			
1994	Jeddah	First detection of autochthonous transmission of DENV in Saudi Arabia;	[29, 40]
	Jeddah	469 cases; DEN-2	[29]
		673 suspected cases, 289 confirmed; DEN-1,2	[30, 41]
1995	n/a	136 suspected cases, 6 confirmed; DEN-1,2	[29, 41]
1996	n/a	57 suspected cases, 2 confirmed	[29]
1997	n/a	62 suspected cases, 15 confirmed; DEN-3	[29, 41]
1998	n/a	31 suspected cases, 0 confirmed	[29]
1999	n/a	26 suspected cases, 3 confirmed	[29]
2000	n/a	17 suspected cases, 0 confirmed	[29]

S4 Table Continued

2001		7 suspected cases, 0 confirmed	[29]
2004	Jeddah, Makkah	DEN-3	[30]
2005	n/a	32 confirmed cases; DEN-3	[29, 30]
2006	n/a	DEN-1	
2006	Jeddah and Makkah	>1,269 cases; DEN-1	[29, 30, 42]
2007	Jeddah and Makkah	200 estimated cases; DEN-3	[30, 42]
2008	Jeddah and Makkah	775 estimated cases; DEN-3	[29, 30, 42]
2009	Jeddah and Makkah	1,500 estimated cases	[42]
2010	Jeddah and Makkah	2,200 estimated cases	[42]
2011	Jeddah and Makkah	2,376 estimated cases	[42]
2012	Jeddah and Makkah	800 estimated cases	[42]
2013	Jeddah and Makkah	4,411 estimated cases	[42]
2006-08	Jeddah	n/a	[43]
2015	Mecca	13 suspected cases	[44]
Somalia			
1941	Mogadishu	72 reported cases	[45]
1982-1983	Mogadishu	DEN-2	[30, 46, 47]
1985-87	Hargeysa	DEN-2	[47, 48]
1992-1993	Mogadishu	41 reported cases; DEN-1,2,3	[30, 49, 50]
2005	n/s	n/s	[51]
2011	Mogadishu	DEN-1,2,3	[52-54]
Sudan			
1984-86	Port Sudan	18 reported cases; DEN-1,2,3	[46, 55-57]
1989	Northern Province	DEN-2	[58]
2003	Port Sudan	366 reported cases	[56, 58]
2004	Multiple	127 reported cases; DEN-1,2	[30, 56]
2005	Multiple	233 reported cases; DEN-1,2	[30, 56]
2006	Multiple	7 reported cases; DEN-1,2	[30, 56]
2008	Multiple, Port Sudan	449 reported cases; DEN-1,2	[30, 56, 59, 60]
2009	Multiple, Port Sudan	447 reported cases	[56, 60]
2010	Port Sudan, multiple	4,008 reported cases; DEN-2	[56]
2011	Multiple	257 reported cases	[56]
2012	Multiple	59 reported cases	[56]
2014	Red Sea State	738 reported cases	[56]
2015	Darfur, Kassala, Kordofan	557 suspected cases	[61]
Yemen			
1983	Dalah	1 case in traveler returning from Yemen	[62]
1994	Shabwah, Al Hudaydah, Mukkala	DEN-3	[30, 63]
2000	Al Hudaydah	653 suspected cases; DEN-2	[14, 30, 63]
2001-2003	Shabwah	DEN-2	[14, 63]
2004	Al Hudaydah, Shabwah	45 suspected cases; DEN-2	[14, 63]
2005-2006	Shabwah, Hadramout, Al Hudaydah	403 suspected cases; DEN-2,3	[14, 63, 64]
2008	Shabwah, Abyan, Taiz, Mukalla	1,001 reported cases; DEN-3	[14, 30, 64]
2009	n/s	900 cases	[64]
2010	Multiple, Hadramout	5,534 suspected cases; DEN-2,3	[64, 65]

S4 Table Continued

2015	Shabwah, Hadramout, Abyan, Maifaa, Eitg, Taiz, Lahj	>20,000 estimated cases	[66-69]
Chikungunya virus			
Djibouti			
2007	Djibouti City	n/a	(c
2011	Djibouti City	CHIKV outbreak reported was concurrent with 2011 outbreak in Yemen	[12]
Saudi Arabia			
2011	Jeddah	first autochthonous case of CHIKV detected in Saudi Arabia	[70]
Sudan			
2005	South Kordofan	concurrent CHIKV transmission detected during YFV outbreak	[71]
Yemen			
2011-12	Al Hudaydah	>15,000 suspected cases	[72]
Yellow Fever virus			
Sudan			
1940	South Kordofan	15,000 suspected cases; first documented YFV outbreak in Sudan	[73]
1959	Blue Nile State	120 reported cases	[74]
2003	Imatong region, South Sudan	178 reported cases	[75, 76]
2005	South Kordofan	615 reported cases	[75]
2012	Darfur	849 reported cases	[77]
2013	South and West Kordofan	49 suspected cases	[74]

S5 Table Summary of precision and risk of bias for dengue (DENV), chikungunya (CHIKV), and yellow fever (YFV) human prevalence measures.

Quality assessment	DENV prevalence measures		CHIKV prevalence measures		YFV prevalence measures	
	n	%	n	%	n	%
Precision of estimates						
High precision	68	69%	15	60%	13	76%
Low precision	31	31%	10	40%	6	24%
Risk of bias quality domains						
Sampling methodology						
Low risk of bias	10	9%	3	8%	3	18%
High risk of bias	85	82%	20	80%	14	82%
Unclear	9	9%	2	12%	0	0%
Response rate						
Low risk of bias	3	3%	1	4%	1	6%
High risk of bias	3	3%	0	0%	0	0%
Unclear	98	94%	23	96%	16	94%
Total number of studies	**104†**		**24†**		**17†**	

* Studies with missing information for any of the domains were classified as having unclear ROB for that specific domain.
† The ROB assessment was performed for all human prevalence measures reported for DENV (Table 1), CHIKV (Table 2), and YFV (Table 3) in the main article.

S6 Table. Precision and risk of bias assessment for dengue virus seroprevalence measures among general populations in the Middle East and North Africa.

Country, Ref.	Year(s) of study*	Population	Sample size	Prevalence	Precision	Sampling	Response rate**
Djibouti							
Salah [78]	1987	healthy soldiers	50	0%	Low	High ROB	Unclear ROB
		general population	69	0%	Low	High ROB	Unclear ROB
Fauld [79]	2011	animal workers	10	10.0%	Low	High ROB	Unclear ROB
Andayi [12]	2010-11	general population	911	21.8%	High	Low ROB	Unclear ROB
Egypt							
Mohammed [80]	1966	general population	29	3.0-7.0%	Low	High ROB	Unclear ROB
		general population	55	4.0-5.0%	Low	High ROB	Unclear ROB
		adults being treated for schistosomiasis	78	0%	Low	High ROB	Unclear ROB
Darwish [81]	1969	university students	1133	0.3%	High	High ROB	Unclear ROB
Iran							
Saidi [82]	1970	n/s	394	6.0%	High	Unclear ROB	Unclear ROB
Saidi [83]	1970-71	children	100	0%	High	High ROB	Unclear ROB
Aghale [84]	2014	blood donors	540	7.6%	High	High ROB	Unclear ROB
Kuwait							
Ibrahim [85]	1966-68	blood donors, patients, children	627	6.5-8.1%	High	High ROB	Unclear ROB
Al-Nakib [86]	1979-82	patients	502	3.2-8.4%**	High	Low ROB	Unclear ROB
Pacsa [87]	2002*	general population	425	13.9%	High	Unclear ROB	Unclear ROB
		general population	47	0%	Low	Unclear ROB	Unclear ROB
		expatriates from South Asia	266	37%	High	Unclear ROB	Unclear ROB
		expatriates from Southeast Asia	31	56.6%	Low	Unclear ROB	Unclear ROB
		expatriates from Middle East	140	25%	High	Unclear ROB	Unclear ROB
Lebanon							
Garabedian [88]	1962-65	general population	113	61.9%	High	Low ROB	Unclear ROB
		general population	171	49.1%	High	Low ROB	Unclear ROB
Hatem [89]	1969	n/s	126	0%	High	Unclear ROB	Unclear ROB
		n/s	126	4.0%	High	Unclear ROB	Unclear ROB
Libya							
Darwish [90]	1973	children, patients	148	0%	High	High RoB	Unclear ROB
Pakistan							
Darwish [4]	1983*	patients	43	9.3%	Low	High ROB	Unclear ROB
Zafar [91]	2009	adults	96	19.8%	Low	Low ROB	Unclear ROB
Zafar [92]	2009	general population	244	28.8%	High	High ROB	Unclear ROB
Saudi Arabia							

S6 Table Continued

Memish [93]	2010	adult military	1024	**0.1%**	High	High ROB	Unclear ROB
Al-Azraqi [94]	2013	patients	268	**26.5%**	High	Low ROB	Unclear ROB
		patients	697	**33.7%**	High	Low ROB	Unclear ROB
Ashshi [95]	2014	blood donors	100	**1-7%**	High	High ROB	Unclear ROB
Somalia							
Sharp [57]	1992-93	adult military	494	**7.7%**	High	High ROB	Low ROB
Nur [96]	1995	children	23	**0%**	Low	High ROB	Unclear ROB
Sudan							
Omer [97]	1976	general population	109	**17.4-27.5%**	High	High ROB	Unclear ROB
McCarthy [98]	1988	patients	100	**49%**	High	High ROB	Unclear ROB
Seidahmed [2]	2008-09	general population	791	**5.2%**	High	Low ROB	Unclear ROB
Himatt [99]	2011	general population	489	**0.6-9.4%**	High	Low ROB	Low ROB
Soghaier [100]	2014	general population	600	**27.7%**	High	Low ROB	Low ROB
Turkey							
Ari [9]	1971	general population	270	**0%**	High	High ROB	Unclear ROB
Radda [101]	1973*	general population	270	**0.3%**	High	High ROB	Unclear ROB
		general population	90	**0%**	Low	High ROB	Unclear ROB
		general population	95	**0%**	Low	High ROB	Unclear ROB
Ergunay [102]	2010	blood donors	2435	**0.9%**	High	High ROB	Unclear ROB
Tezcan [103]	2010-11	blood donors	920	**0.9-16.6%**	High	High ROB	Unclear ROB

S7 Table. Precision and risk of bias assessment for dengue virus prevalence measures among acute febrile illness populations and those with suspected dengue infection in the Middle East and North Africa.

Country, Ref.	Year(s) of study	Setting; population	Sample size	Prevalence	Precision	Sampling	Response rate
Afghanistan							
Elyan [104]	2008-10	AFI patients	913	**19.2%**	High	High ROB	Unclear ROB
Djibouti							
Salah [78]	1987	AFI patients	41	**0%**	Low	High ROB	Unclear ROB
Rodier [13]	1991	AFI patients	91	**7.7-25.0%**	Low	High ROB	Unclear ROB
Egypt							
Mohammed [105]	1968	AFI patients	120	**0%**	High	High ROB	Unclear ROB
Iran							
Chinikar [106]	2000-12	AFI patients	300	**1.0-3.3%**	High	High ROB	Unclear ROB
Kuwait							
Pacsa [87]	2002*	suspected dengue	210	**9.0%**	High	Unclear ROB	Unclear ROB
Pakistan							
Akram [107]	1994	AFI patients	122	**9.8-14.6%**	High	High ROB	Unclear ROB
Siddiqui [1]	1999-2001	AFI patients	341	**15.8%**	High	High ROB	High ROB
Jamil [108]	2005	suspected dengue	106	**36.8%**	High	High ROB	Unclear ROB
Khan [109]	2006	suspected dengue	83	**50.7-87.9%**	Low	High ROB	Unclear ROB
Khan [110]	2006	suspected dengue	250	**23.2-74%**	High	High ROB	Unclear ROB
Koo [111]	2006-11	suspected dengue	200	**47%**	High	High ROB	Unclear ROB
Khan [112]	2006-07	suspected dengue	50	**40%**	Low	High ROB	Unclear ROB
Khan [113]	2006-07	suspected dengue	15,040	**26.3%**	High	High ROB	Unclear ROB
Abbasi [114]	2007-08	suspected dengue	114	**69.6%**	High	High ROB	Unclear ROB
Murad [115]	2008	suspected dengue	70	**17.1%**	Low	High ROB	Unclear ROB
Kidwai [116]	2008-09	suspected dengue	599	**41.9-83.2%**	High	High ROB	Unclear ROB
Hasan [117]	2010	suspected dengue	259	**34.8%**	High	High ROB	Unclear ROB
Umar [118]	2010	suspected dengue	500	**6.8%**	High	High ROB	Unclear ROB
Ahmed [36]	2011	suspected dengue	640	**43.9%**	High	High ROB	Unclear ROB
Ijaz [119]	2011	suspected dengue	5,274	**49%**	High	High ROB	Unclear ROB
Khan [24]	2011	suspected dengue	50	**60-72%**	Low	High ROB	Unclear ROB
Hasan [120]	2007-13	suspected Crimean-Congo Hemorrhagic Fever	168	**33.9%**	High	High ROB	Unclear ROB
Ali [121]	2011	suspected dengue	612	**20.2%**	High	High ROB	Unclear ROB
Hisam [122]	2012	AFI patients	500	**3.2%**	High	Low ROB	Unclear ROB
Assir[123]	2012	suspected dengue	85	**43.5%**	Low	Low ROB	Unclear ROB
Saudi Arabia							
Fakeeh [124]	1994-99	suspected dengue	985	**16.2-31.9%**	High	High ROB	Unclear ROB
Fakeeh [125]	1994-2002	suspected dengue	1020	**10.8-50.5%**	High	High ROB	Unclear ROB
Khan [126]	2004	suspected dengue	136	**32.4-58.8%**	High	High ROB	Unclear ROB
Ayyub [127]	2004-05	suspected dengue	80	**48.8%**	High	High ROB	Unclear ROB

S7 Table Continued

Shahin [128]	2006-08	suspected dengue	159	**100%**	High	High ROB	Unclear ROB
Gamil [129]	2010-11	suspected dengue	553	**47.7%**	High	High ROB	Unclear ROB
Somalia							
Botros [47]	1987	AFI patients	38	**14.2-60.7%**	Low	High ROB	Unclear ROB
Kanesa-thasan [130]	1993	AFI patients	84	**17.8%**	Low	High ROB	Unclear ROB
Sharp [57]	1992-93	AFI patients	96	**2-40.6%**	Low	High ROB	High ROB
		AFI patients	129	**34.9%**	High	High ROB	High ROB
Nur [96]	1995	AFI patients	46	**0%**	Low	High ROB	Unclear ROB
Kyobe Bosa [53]	2011	AFI patients	134	**62-80%**	High	High ROB	Unclear ROB
Sudan							
Hyams [131]	1984	AFI patients	100	**21%**	High	High ROB	Unclear ROB
Woodruff [132]	1986	AFI patients	130	**40.0%**	High	High ROB	Unclear ROB
McCarthy [98]	1988	AFI patients	196	**48%**	High	High ROB	Unclear ROB
Watts [133]	1989	AFI patients	185	**24.0%**	High	High ROB	Unclear ROB
Ibrahim [134]	1997-99	suspected measles	188	**3.2%**	High	High ROB	Unclear ROB
Malik [58]	2004-05	Hospitals; suspected dengue	40	**90.0%**	Low	High ROB	Unclear ROB
Farnon [135]	2005	AFI patients	87	**1.1%**	Low	Low ROB	Unclear ROB
Gould [71]	2005	AFI patients	34	**5.9%****	Low	High ROB	Unclear ROB
Adam [60]	2008-09	pregnant women	10,820	**0.7%**	High	High ROB	Unclear ROB
Abdalla [136]	2012	AFI patients	60	**11.7%**	Low	High ROB	Unclear ROB
Elduma [137]	2012	pregnant women with AFI	39	**12.8%**	Low	High ROB	Unclear ROB
Yemen							
Bin Ghouth [138]	2011	suspected dengue	982	**50.6-64.1%**	High	High ROB	Unclear ROB
Malik [139]	2010-11	AFI patients	136	**8.1-87.5%**	High	High ROB	Unclear ROB
Madani [65]	2010	suspected viral hemorrhagic fever	207	**0.9-78.7%**	High	High ROB	Unclear ROB
Rezza [140]	2012	suspected dengue	400	**13.8%**	High	High ROB	Unclear ROB*
Qassem [141]	2013	suspected dengue or west nile fever	42	**19.0%**	Low	High ROB	Unclear ROB

S8 Table. Precision and risk of bias assessment for chikungunya virus seroprevalence measures among general populations in the Middle East and North Africa.

Country, Ref.	Year(s) of study*	Population	Sample size	Prevalence	Precision	Sampling	Response rate**
Djibouti							
Salah [78]	1987	healthy soldiers	50	**0%**	Low	High ROB	Unclear ROB
		general population	69	**0%**	Low	High ROB	Unclear ROB
Andayi [12]	2010-11	general population	914	**2.6%**	High	Low ROB	Unclear ROB
Egypt							
Darwish [142]	1974*	general population	231	**0%**	High	Unclear ROB	Unclear ROB
Iran							
Saidi [82]	1970	n/s	394	**22.1%**	High	Unclear ROB	Unclear ROB
Saidi [83]	1970-71	children	100	**0%**	High	High ROB	Unclear ROB
Kuwait							
Ibrahim [143]	1966-68	blood donors, non-AFI patients, children	627	**1.4%**	High	High ROB	Unclear ROB
Al-Nakib [86]	1979-82	non-AFI patients	502	**0.4%**	High	Low ROB	Unclear ROB
Pakistan							
Darwish [4]	1983*	Hospital patients	43	**2.3%**	Low	High ROB	Unclear ROB
Sudan							
Salim [144]	1973*	general population and non-AFI patients	62	**12.9%**	Low	High ROB	Unclear ROB
Omer [97]	1976	general population	109	**24.8%**	High	High ROB	Unclear ROB
McCarthy [98]	1988	non-AFI patients	100	**11%**	High	High ROB	Unclear ROB
Farnon [135]	2005	general population	84	**43%**	Low	Low ROB	Low ROB
Tunisia							
Nabil [145]	1970*	children	100	**0%**	High	High ROB	Unclear ROB

S9 Table. Precision and risk of bias assessment for chikungunya virus seroprevalence measures among acute febrile illness populations in the Middle East and North Africa.

Country, Ref.	Year(s) of study*	Population	Sample size	Prevalence	Precision	Sampling	Response rate**
Djibouti							
Salah [78]	1987	AFI patients	41	**2.4%**	Low	High ROB	Unclear ROB
Egypt					Low		
Darwish [146]	1985	AFI patients	55	**5.5%**	High	High ROB	Unclear ROB
Somalia							
Botros [47]	1987	AFI patients	28	**n/a**	Low	High ROB	Unclear ROB
Sudan							
Woodruff [132]	1986	AFI patients	130	**23.1%**	High	High ROB	Unclear ROB
McCarthy [98]	1988	AFI patients	196	**10%**	High	High ROB	Unclear ROB
Watts [133]	1989	AFI patients	185	**12.0%**	High	High ROB	Unclear ROB
Gould [71]	2005	AFI patients	34	**23.5%**	Low	High ROB	Unclear ROB
Yemen							
Malik [139]	2010-11	AFI patients	136	**22-40%**	High	High ROB	Unclear ROB
Madani [65]	2010	suspected viral hemorrhagic fever	207	**0%**	High	High ROB	Unclear ROB
Rezza [147]	2012	patients with dengue-like illness	400	**2.5-9.5%**	High	High ROB	Unclear ROB

S10 Table. Precision and risk of bias assessment for yellow fever virus seroprevalence measures among general populations in the Middle East and North Africa.

Country, Ref.	Year(s) of study*	Population	Sample size	Prevalence	Precision	Sampling	Response rate**
Djibouti							
Salah [78]	1987	healthy soldiers	50	**0%**	Low	High ROB	Unclear ROB
		general population	4169	**0%**	High	High ROB	Unclear ROB
Andayi [12]	2010-11	general population	903	**1.6%**	High	Low ROB	Unclear ROB
Egypt							
Smithburn [148]	1954*	general population	331	**0%**	High	Low ROB	Unclear ROB
Lebanon							
Garabedian [88]	1962-65	general population	115	**61.7%****	High	Low ROB	Unclear ROB
Somalia							
Henderson [149]	1966	general population	242	**0-53.3%****	High	High ROB	Unclear ROB
Sudan							
Taylor [6]	1952-54	general population	666	**14.5%**	High	High ROB	Unclear ROB
Salim [144]	1973	general population and non-AFI patients	62	**95.1%**	Low	High ROB	Unclear ROB
Turkey							
Ergunay [102]	2010*	blood donors	1502	**0.6%**	High	High ROB	Low ROB

S11 Table. Precision and risk of bias assessment for yellow fever virus seroprevalence measures among acute febrile illness populations in the Middle East and North Africa.

Country, Ref.	Year(s) of study*	Population	Sample size	Prevalence	Precision	Sampling	Response rate
Djibouti							
Salah [78]	1987	AFI patients	41	0%	Low	High ROB	Unclear ROB*
Rodier [13]	1991	AFI patients	91	10.9%	Low	High ROB	Unclear ROB*
Egypt							
Mohammed [105]	1968	AFI patients	120	0%	High	High ROB	Unclear ROB*
Somalia							
Botros [47]	1987	AFI patients	28	0%	Low	High ROB	Unclear ROB*
Sudan							
Woodruff [132]	1986	AFI patients	130	0.8%	High	High ROB	Unclear ROB*
Watts [133]	1989	AFI patients	185	39%	High	High ROB	Unclear ROB*
Gould [71]	2005	AFI patients	34	17.6%	Low	High ROB	Unclear ROB*
Yemen							
Madani [65]	2010	patients with suspected viral hemorrhagic fever	207	0%	High	High ROB	Unclear ROB*

CHAPTER / MANUSCRIPT 2

TITLE PAGE

Multiplex PCR for Detection of Gastrointestinal Pathogens In Migrant Workers in Qatar

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ABSTRACT

The causes of infectious diarrhea among the migrant worker population in Qatar are not well understood. We conducted a prospective observational study to understand the demographic and clinical characteristics and infectious causes of diarrhea among migrant workers in Doha, Qatar. Seventy-five male workers coming to the Qatar Red Crescent Worker's Health Center outpatient clinic or emergency department were enrolled over a three-month period in 2015-2016. Epidemiologic surveys were administered to all subjects and the prevalence of 23 different stool pathogens was determined using multiplex PCR (FilmArray® Gastrointestinal PCR). A target pathogen was identified in 57% of subjects. *Salmonella* was the most prevalent pathogen and was detected in 27% of all subjects, followed by enteropathogenic *E. coli* (21%), enteroaggregative *E. coli* (15%) and enterotoxigenic *E. coli* (12%). Co-detection of multiple pathogens was identified in 54% of positive stool samples. In an multivariable analysis, a triage heart rate ≥ 90 beats per minute was the only significant predictor of a positive PCR result (OR 4.5, 95% CI 1.1-18.7). Use of multiplex PCR enabled the detection of gastrointestinal pathogens in a high proportion of cases, illustrating the utility of this diagnostic tool in epidemiologic studies of infectious diarrhea.

INTRODUCTION

Infectious diarrhea is an important cause of morbidity and mortality worldwide, resulting in considerable economic and public health burdens in both developed and developing countries [1-4]. In Qatar and the greater Gulf

region, few studies have been undertaken to characterize the epidemiology of infectious diarrhea [5, 6]. This is particularly the case among Qatar's migrant worker population, over half a million of which are young and middle aged men from the Indian subcontinent and other Asian countries who have come to work in Qatar's surging construction sector [7]. Many of these individuals live in populous labor camps with dormitory style housing which pose risk for transmission of communicable diseases, including gastrointestinal pathogens. Indeed, data from Qatar's Ministry of Public Health suggest that a large proportion of the country's annual foodborne disease outbreaks occur in this community (Farag, E.A., personal communication). Concerns have been raised over the possibility of underdetection of foodborne disease outbreaks in the migrant worker community and over the possibility of imported gastrointestinal pathogens. In addition, the unprecedented mass gathering anticipated during 2022 World Cup further motivates the need to understand the current epidemiology of diarrheal diseases in the country.

Recently, multiplexed molecular diagnostics have become commercially available for the diagnosis of infectious diarrhea. These assays offer high sensitivity and specificity for a range of pathogens, providing a novel opportunity to narrow the diagnostic gap concerning the causes of infectious diarrhea [1, 8]. One such technology, the FilmArray® Gastrointestinal PCR is capable of detecting 23 different pathogens with a sensitivity ranging from 94.5-100% and specificity from 97.1-100% depending on the target [9]. Such platforms are well suited for settings such as the migrant worker community in Qatar, for which broad diagnostic capacity near the point of care is currently

needed to facilitate actionable data concerning the causes of potential foodborne disease outbreaks [9, 10].

Given the need for further data concerning the epidemiology of infectious diarrhea in the migrant worker population in Qatar, we conducted a prospective observational study aimed at describing the clinical features, epidemiologic characteristics, and etiologies of infectious diarrhea in this population using the FilmArray® Gastrointestinal PCR.

MATERIALS AND METHODS

Study site and ethical approval. This prospective, clinic-based study was conducted at the Qatar Red Crescent Workers Health Center (QRC), located in the New Industrial Area of Doha, Qatar. This facility provides outpatient and emergency department (ED) care exclusively to male migrant laborers, attending to 800 to 1,000 patients per day. This study was approved by the Institutional Review Board at Weill Cornell Medical College in Qatar (IRB #15-00051). All study participants provided written, informed consent.

Enrollment procedures and inclusion/exclusion criteria. From August-September 2015 and January-March 2016, any individual coming to the clinic or ED with suspected infectious diarrhea was eligible to participate in the study. To be eligible for enrollment, subjects had to be ≥ 18 years of age and able to understand English, Arabic, Hindi, Malayalam, or Tagalog. These languages represented the majority of the clinic population and were based on the language capacity of the study personnel. For the study, presumptive

infectious diarrhea was defined as 3 or more loose stools in a 24-hour period or two loose stools in a 24-hour period accompanied by other gastrointestinal symptoms such as nausea, vomiting, abdominal cramps, tenesmus, bloody stools, or fever, which was defined as an oral temperature ≥ 38ºC [6, 11]. Subjects were excluded from the study if they were diagnosed with a non-infectious cause of diarrhea, unable to provide a stool sample or complete the survey, or if the sample provided was not formed stool (i.e. did not conform to the shape of the collection container). Of note, subjects were asked to submit a stool sample for PCR testing regardless of whether stool culture or microscopy was ordered by the subject's physician. We aimed to enroll 200 subjects in order to detect a *Salmonella* prevalence of 5% with 95% confidence intervals of 2.4-9.0% [5, 9, 12].

Demographic/clinical data collection and microbiological analysis. A member of the study team administered a survey to enrolled subjects that assessed their demographic and clinical characteristics and a variety of risk factors for infectious diarrhea. The survey was an adaptation of the Minnesota Questionnaire, a standard foodborne disease outbreak case questionnaire (Appendix 2), and translated into the above study languages using a certified translation service (Language Scientific, Medford, MA, USA) [13]. Additional clinical and laboratory data (i.e. triage vital signs, medications prescribed, and pertinent laboratory results) were recorded retrospectively by accessing the patient's medical record. These data were entered into a Research Electronic Data Capture (RedCap) database [14]. Stool samples were collected from study participants at the QRC clinic and immediately preserved in Cary-Blair enteric transport medium. The samples were then transported to Hamad

General Hospital Microbiology Laboratory in Doha, Qatar within 1-2 days, where they were tested with a commercially available multiplex PCR system, the FilmArray® Gastrointestinal Panel, (Biofire Diagnostics, Salt Lake City, UT, USA). The multiplex PCR was validated according to the manufacturer instructions prior to beginning the study. During the study, synthetic RNA quality controls were run for every 20 samples tested (Maine Molecular Quality Controls, Scarborough, ME, USA). Due to the time required to transport the specimens to the facility where they were tested, PCR results were typically reported to the subject's physician within 2-4 days. Hence, treatment recommendations were made empirically by the subject's physicians at the time of the visit.

Statistical analyses. Associations between various demographic and clinical variables and the detection of any pathogen by PCR were summarized with odds ratios and 95% confidence intervals. Backward stepwise regression was used to construct a multivariable model to identify factors predictive of a positive PCR result for any pathogen. The following factors were included in the model: nationality, subjective fever, vomiting, temperature ≥38.5^{o}C, heart rate ≥ 90 beats per minute, ≥6 stools in a 24-hour period, >5 fecal leukocytes/high-power field [16], ≥1 fecal red blood cell, and treatment in the emergency department. These factors were selected for conceptual reasons with a probability of removal from the model (P_r) set at 0.2. Data were analyzed in STATA 14.1 (StataCorp, College Station, TX).

RESULTS

Enrollment. A total of 92 subjects were enrolled into the observational study. After enrollment, 17 subjects were excluded because they did not submit a stool sample (n=2), submitted formed stool (n=13), or submitted an insufficient quantity of stool for PCR testing (n=2). Thus, 75 subjects who completed the survey and submitted unformed stool were included in the analysis.

Demographic characteristics. Table 2.1 summarizes the demographic characteristics of the study subjects. All subjects were male and the median age was 33 years. All subjects were migrants, most were from the Indian subcontinent, and 8% reported having returned to Qatar from their home country within 7 days of clinical presentation for diarrhea. Nearly all subjects were employed in construction-related fields and were living in dormitories within worker camps, each individual sharing a room and bathroom with a median of 5 other individuals.

Table 2.1 Demographic characteristics of study participants

Table 2.1 The demographic characteristics of the study participants.

Characteristic	n (%)*
Median age (IQR) in years	33 (27-39)
Male sex	75 (100)
Country of origin	
Nepal	24 (32.0)
India	20 (26.7)
Sri Lanka	12 (16.0)
Bangladesh	11 (14.7)
Philippines	5 (6.7)
Kenya	1 (1.3)
Ethiopia	1 (1.3)
Syria	1 (1.3)
Type of work	
construction	13 (17.3)
carpentery	11 (14.7)
plumber/pipefitter	8 (10.7)
metal worker/welder	8 (10.7)
electrician	7 (9.3)
painter	7 (9.3)
other[a]	21 (28)
Shift time	
day	72 (96)
night	2 (2.7)
not specified	1 (1.3)
Dwelling	
worker camp	72 (96)
private apartment	2 (2.7)
private house	1 (1.3)
Number of roommates	
1-3	17 (23.0)
4-6	31 (41.9)
7-10	24 (32.4)
11-13	2 (2.7)
Shared bathroom	74 (98.7)
International travel ≤ 7 days of presentation	6 (8.0)[b]

*Data are reported as n (%) except where otherwise indicated

IQR=interquartile range

[a] Other work types include helper/cleaner (n=5), mason (n=3), machine operator (n=3), foreman (n=3), driver (n=2), store keeper (n=2), mechanic (n=1), chemical sprayer (n=1), insulation installer (n=1)

[b] Countries traveled to include India (n=3), Bangladesh (n=2), Nepal (n=1)

Clinical characteristics. Table 2.2 summarizes the clinical characteristics of study participants. Overall, 24% of subjects were evaluated and treated in the emergency department. Two-thirds of individuals rated their baseline health as excellent or very good. Seven (9.4%) reported a medical co-morbidity, most frequently diabetes, and 4 (5.3%) reported a remote history of intra-abdominal surgery (appendectomy in all cases). No subjects were taking acid-suppressive or immunosuppressive medications at the time of presentation, and 1.3% reported taking an antibiotic for any reason prior to developing diarrhea. The median duration of symptoms prior to clinical presentation was 2 days, and 43% of subjects reported a maximum of ≥ 6 stools in a 24-hour period, a cutoff suggested as an indicator of severe diarrhea [15]. A total of 29.8% of evaluable subjects had a heart rate ≥ 90 beats per minute and 10.6% had a temperature ≥ 38.5^{o}C.

Table 2.2 Clinical characteristics of study participants.

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Characteristic	n (%)*
Self-rated baseline health	
Excellent	34 (45.3)
Very good	18 (24.0)
Good	14 (18.7)
Fair	7 (9.3)
Poor	2 (2.7)
Current medical comorbidity	7 (9.4)[a]
History of intra-abdominal surgery	4 (5.3%)[b]
Antibiotic use prior to diarrheal illness (n=73)	
yes	1 (1.3)
no	69 (94.6)
unknown	3 (4.1)
Contact with another person with diarrheal illness	3 (4.0)
Acid-suppressive or immunosuppressive medication use	0 (0)
Location of treatment	
clinic	57 (76.0)
emergency department	18 (24.0)
Median duration of symptoms (IQR) in days	2 (1-3)
Symptoms	
diarrhea	75 (100.0)
abdominal cramps	59 (78.7)
fatigue	59 (78.6)
fever	37 (49.3)
chills	32 (42.7)
headache	34 (45.3)
body aches	34 (45.3)
nausea	22 (29.3)
vomiting	19 (25.3)
bloody diarrhea	2 (2.7)
≥6 stools per day (n=74)	32 (43.2)
Temperature ≥ 38.5^{o}C (n=65)	5 (7.7)
Heart rate ≥ 90 beats per minute (n=47)	17 (29.8)
Systolic blood pressure < 90 mmHg (n=35)	0

*Data are reported as n (%) except where otherwise indicated

IQR=interquartile range

[a] Medical comorbidity includes diabetes (n=4), hyperlipidemia (n=1); arthritis (n=1), hypertension (n=2)

[b] All had undergone appendectomy years prior to presentation

Laboratory and Stool PCR Results. Table 2.3 summarizes the available laboratory data. Complete blood counts were performed in 11 subjects, of whom 4 (36.6%) had white blood cell counts ≥ 15,000 per mm^3. No subjects had low hematocrit or platelet values. Stool microscopy was performed in all subjects. One or more fecal leukocytes were present in 94.2% of all stool samples and *Entamoeba sp*. was identified in 15.3% of all samples by microscopy. Stool culture was ordered for 5 subjects and was negative in all cases. Figure 2.1 depicts the prevalence of pathogens detected by multiplex PCR. Overall, one or more pathogens was identified in 57% of samples. *Salmonella sp*. was the most prevalent pathogen and was identified in 27% of samples. Diarrheagenic *E. coli* strains were also prevalent, including Enteropathogenic *E. coli* (EPEC) in 21%, Enteroaggregative *E. coli* (EAEC) in 15%, and Enterotoxigenic *E. coli* (ETEC) in 12% of samples. Norovirus was identified in 5% of samples and *Clostridium difficile* was not identified in any samples. Two or more PCR targets were detected in 53% of positive samples (Table 2.3). Co-detections occurred for the majority of all pathogens with the exception of *Salmonella*, for which co-detection of multiple pathogens was identified in 35% of samples (Figure 2.1). The presence of a heart rate ≥ 90 beats per minute was the only significant predictor of a positive PCR result in the univariate analysis. This covariate remained a significant predictor (OR 4.5, 95% CI 1.1-18.7) when adjusted for the aforementioned covariates in the stepwise multivariable analysis.

Table 2.3 Laboratory and microbiology test results of study participants.

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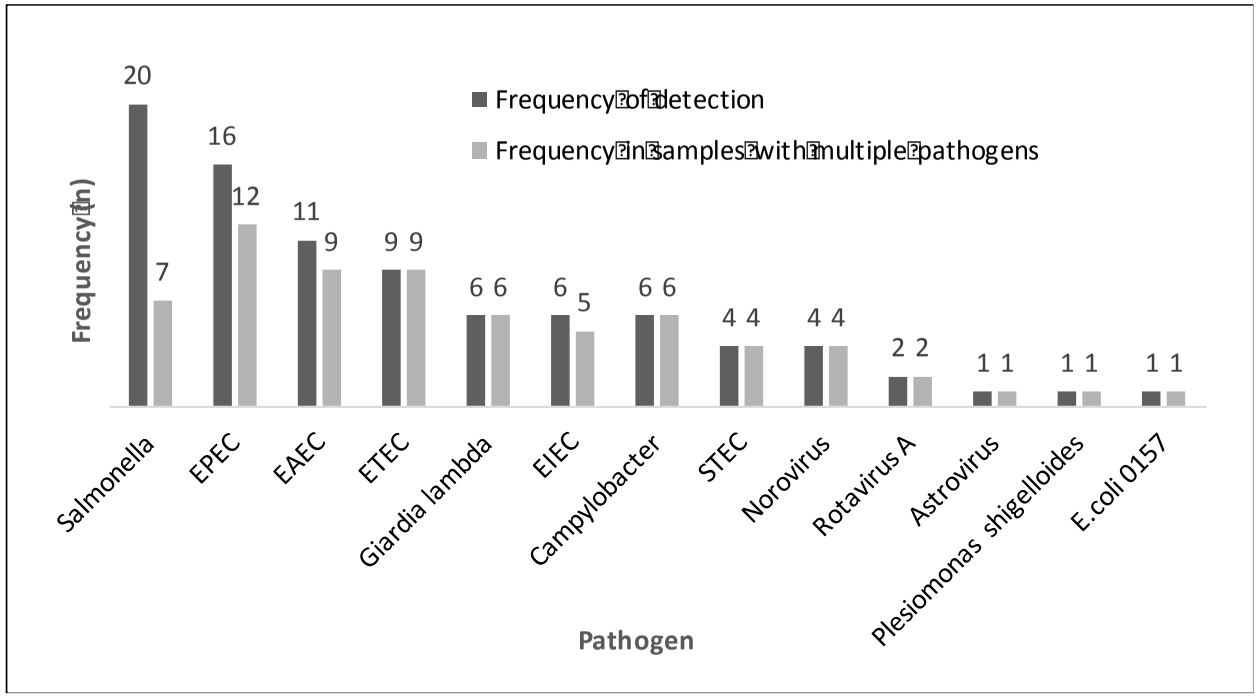
Characteristic	n (%)
Blood count (n=11)	
white blood cells ≥15,000 per mm^3 (n=11)	4 (36.6)
hematocrit < 40%	0 (0)
platelet <150,000 per microliter	0 (0)
Stool microscopic exam (n=52)	
fecal leukocytes present	49 (94.2)
>5 leukocytes/high-power field	36 (69.2)
red blood cells present	16 (30.7)
Entamoeba sp.	8 (15.3)
Giardia lamblia	1 (1.9)
Enterobius vermicularis	1 (1.9)
Ascaris lumbricoides	1 (1.9)
hookworm	1 (1.9)
Stool culture for *Salmonella/Shigella* (n=5)	
negative	5 (100.0)
FilmArray® Gastrointestinal PCR (n=75)	
negative	32 (42.7)
positive	43 (57.3)
Pathogen Frequency	
Salmonella	20 (46.5)
Enteropathogenic *E. coli* (EPEC)	16 (37.2)
Enteroaggregative *E. coli* (EAEC)	11 (25.6)
Enterotoxigenic *E. coli* (ETEC) lt/st	9 (20.9)
Shigella/Enteroinvasive *E. coli* (EIEC)	6 (13.9)
Campylobacter	6 (13.9)
Giardia lambda	6 (13.9)
Shiga-like toxin-producing *E. coli* (STEC) stx1/stx2	4 (9.3)
Norovirus	4 (9.3)
Rotavirus A	2 (4.7)
Astrovirus	1 (2.3)
Plesiomonas shigelloides	1 (2.3)
E.coli 0157	1 (2.3)
Number of pathogens per positive sample (n=43)	
1	20 (46.5)
2	12 (27.9)
3	5 (11.7)
4	4 (9.3)
5	1 (2.3)
6	0 (0)
7	1 (2.3)
Received antibiotic (n=66)	36 (65.4)
metronidazole	27 (43.6)
ciprofloxacin	24 (38.1)
ciprofloxacin + metronidazole	9 (13.6)
trimethoprim-sulfamethoxazole	2 (3.0)
Received IV fluids (n=55)	12 (18.8)
Discharged from clinic or emergency department	71 (94.7)
Hospitalized	2 (3.0)

Figure 2.1 Frequency of pathogen detection by multiplex PCR and frequency of detection in samples with ≥ 2 pathogens.



Abbreviations: EAEC, enteroaggregative *E. coli*; EIEC, *Shigella*/enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli;* STEC, shiga-like toxin-producing *E. col*

Figure 2.1 Frequency of pathogen detection by multiplex PCR and frequency of detection in samples with ≥ 2 pathogens.

Treatment and outcome. A total of 65% of evaluable subjects received an antibiotic, most commonly metronidazole (43%), ciprofloxacin (38%) or both in combination (13%) (Table 2.3). An antibiotic was prescribed in 15% of those for whom PCR was negative for all pathogens. Moreover, 5 (27%) of individuals who received ciprofloxacin did not have any potentially susceptible bacterial pathogen detected by PCR (i.e. *Campylobacter*, *Salmonella*, *Shigella*, or any *E. coli*), and among those prescribed metronidazole, *Entamoeba histolytica* or *Giardia lamblia* was not detected in 85% (18 of 21 cases) (Table 2.4). A total of 3% of subjects were hospitalized for continued treatment directly after initial treatment at the study center.

Table 2.4 Prevalence of various demographic and clinical characteristics in patients with positive and negative multiplex PCR results.

Table 2.4 Prevalence of various demographic and clinical characteristics in patients with positive and negative multiplex PCR results.

Variable	Positive PCR n (%) (n=43)	Negative PCR n (%) (n=32)	OR (95% CI)
Demographic			
Country			
India	14 (32.6)	6 (18.8)	2.0 (0.6-7.5)
Nepal	12 (27.9)	12 (37.5)	0.6 (0.2-1.9)
Bangladesh	5 (11.6)	6 (18.8)	0.5 (0.1-2.5)
Philippines	3 (7.0)	2 (6.3)	0.3 (0.04-1.7)
Sri Lanka	8 (18.6)	4 (12.5)	1.6 (0.3-7.9)
International travel ≤ 7 days of presentation	4 (9.3)	2 (6.3)	1.5 (0.2-17.9)
living with ≥ 6 roommates	17 (39.5)	9 (28.1)	1.7 (0.5-5.3)
Meal location			
home	27 (62.8)	22 (68.8)	0.7 (0.2-2.2)
workplace cafeteria	4 (9.3)	1 (3.1)	3.1 (0.2-161.5)
catering company	7 (16.3)	9 (28.1)	0.4 (0.1-1.7)
cooking stove in home	33 (76.7)	20 (62.5)	1.8 (0.5-5.7)
refrigerator in home	23 (53.5)	16 (50.0)	1.1 (0.4-3.0)
Clinical			
Location of treatment			
emergency department	12 (27.9)	6 (18.8)	1.6 (0.4-6.2)
Symptom duration ≤ 1 day	32 (74.4)	20 (62.5)	1.4 (0.4-4.5)
Symptoms			
abdominal cramps	36 (83.7)	23 (71.9)	2.0 (0.5-7.2)
fatigue	33 (76.7)	26 (81.2)	0.76 (0.2-2.6)
fever	19 (44.1)	19 (59.3)	0.5 (0.1-1.5)
chills	20 (46.5)	12 (37.5)	1.4 (0.5-4.1)
headache	23 (53.5)	11 (34.3)	2.1 (0.7-6.3)
body aches	23 (53.5)	11 (34.3)	2.1 (0.7-6.3)
nausea	16 (37.2)	6 (18.8)	2.5 (0.6-9.1)
vomiting	14 (32.6)	5 (15.6)	2.6 (0.7-10.4)
bloody diarrhea	2 (4.7)	0 (0)	NC
≥6 stools per day (n=74)	21 (50.0)	11 (34.3)	1.9 (0.6-5.5)
Temperature ≥ 38.5^{o}C (n=47)	3 (11.1)	2 (10.0)	1.1 (0.1-0.6)
Heart rate ≥ 90 beats per minute (n=57)	14 (41.1)	3 (13.0)	4.6 (1.0-28.4)*
Laboratory data (n=64)			
> 5 stool leukocytes	14 (37.8)	11 (40.7)	0.8 (0.2-2.7)
≥ 1 stool red blood cell	14 (37.8)	10 (37.0)	1.0 (0.3-3.4)
Treatment (n=66)			
receipt of any empiric antimicrobial	28 (73.6)	16 (57.1)	2.1 (0.6-6.8)

* indicates significant association

Abbreviations: CI, confidence interval; NC, not calculated; OR, odds ratio; PCR polymerase chain reaction

DISCUSSION

Our study offers a detailed picture of the epidemiology of infectious diarrhea in the migrant worker population in Qatar. We found that *Salmonella* accounts for a high proportion of diarrhea cases, followed by the diarrheagenic *E. coli* pathotypes EPEC, EAEC, and ETEC. Most of these infections would not have been ascertained under the present clinical and laboratory practices at the study site. In addition, the high overall pathogen detection rate of 57% in our study demonstrates the utility of multiplex PCR in epidemiologic surveillance studies and its potential to influence antibiotic prescribing practices for diarrheal diseases.

In many settings, the etiologies of diarrheal diseases remain poorly characterized due to limited surveillance and the low detection rates of traditional pathogen identification methods [1, 2, 17]. In our study using multiplex PCR, 11 different pathogens were identified with an overall detection rate of 57%. This detection rate exceeds traditional laboratory detection methods [2] and compares favorably to prior studies utilizing the FilmArray® PCR in which detection rates ranged from 33-53% [9, 12, 18]. Thus, the breadth of pathogens surveyed and ease of use of this PCR platform make it well suited for epidemiologic studies, particularly in settings such as ours in which the traditional suite of culture media types, immunoassays and uniplex PCR protocols used to survey a similarly broad range of pathogens would be difficult to implement due to limited human and laboratory resources.

Another notable finding in our study was the 27% prevalence of *Salmonella* encountered in the study cohort. This prevalence exceeds those reported from cohorts of similar ages in Qatar [5] and elsewhere [9, 12, 17, 19, 20], where *Salmonella* prevalence ranged from 2-10% in both PCR and culture-based studies. Nontyphoidal *Salmonella* is recognized as a major bacterial cause of infectious diarrhea and the most common bacterial cause of foodborne outbreaks, but its incidence has declined over recent years in both Europe [21] and the United States [22]. In our study, whether the *Salmonella* cases were sporadic or outbreak-related could not be determined given the absence of culture isolates for most cases. However, the temporal pattern and the diverse demographic characteristics of *Salmonella* cases suggested that these cases were sporadic. Nevertheless, this finding warrants further research to clarify the epidemiology and risk factors for *Salmonella* infections in the migrant worker population and to evaluate to need for targeted prevention measures.

Diarrheagenic *E. coli* pathotypes were also commonly detected in our study population. This finding is consistent with prior surveillance studies utilizing multiplex PCR [9, 12, 23]. Unlike studies in other settings, however, ours detected a relatively low frequency of norovirus and no cases of *C. difficile*. Our study definition may have influenced this finding, as not including vomiting as a standalone inclusion criterion may have reduced the number of norovirus cases we were able to enroll. The absence of *C. difficile* is not unexpected given the study population of predominantly young, healthy adults with limited prior antibiotic exposure or contact with healthcare environments where *C. difficile* is prevalent.

In our study, two or more pathogens were identified in 53% of positive samples and co-detections were present in the majority of pathogens detected except for *Salmonella* (Figure 2.2). The high frequency of co-detection for EPEC, ETEC, and EAEC is consistent with prior studies [12, 18]. Still, co-detections were reported in 16-31% of positive samples in prior studies using the FilmArray® PCR [9, 12, 18], all of which exceeded the co-detection rates of routine comparator methods. Whether stools containing multiple pathogens represent true co-infections or transient colonizers is unclear [15]. Nevertheless, the identification of multiple-pathogen-containing stool specimens will likely become more common with the broader clinical use of highly multiplexed molecular diagnostics. The high co-detection rate in our study also raises the potential importance of investigating the role of co-pathogens in future studies of pathogen-specific enteric gastrointestinal infections.

Figure 2.2 Results of multiplex PCR testing for positive stool samples

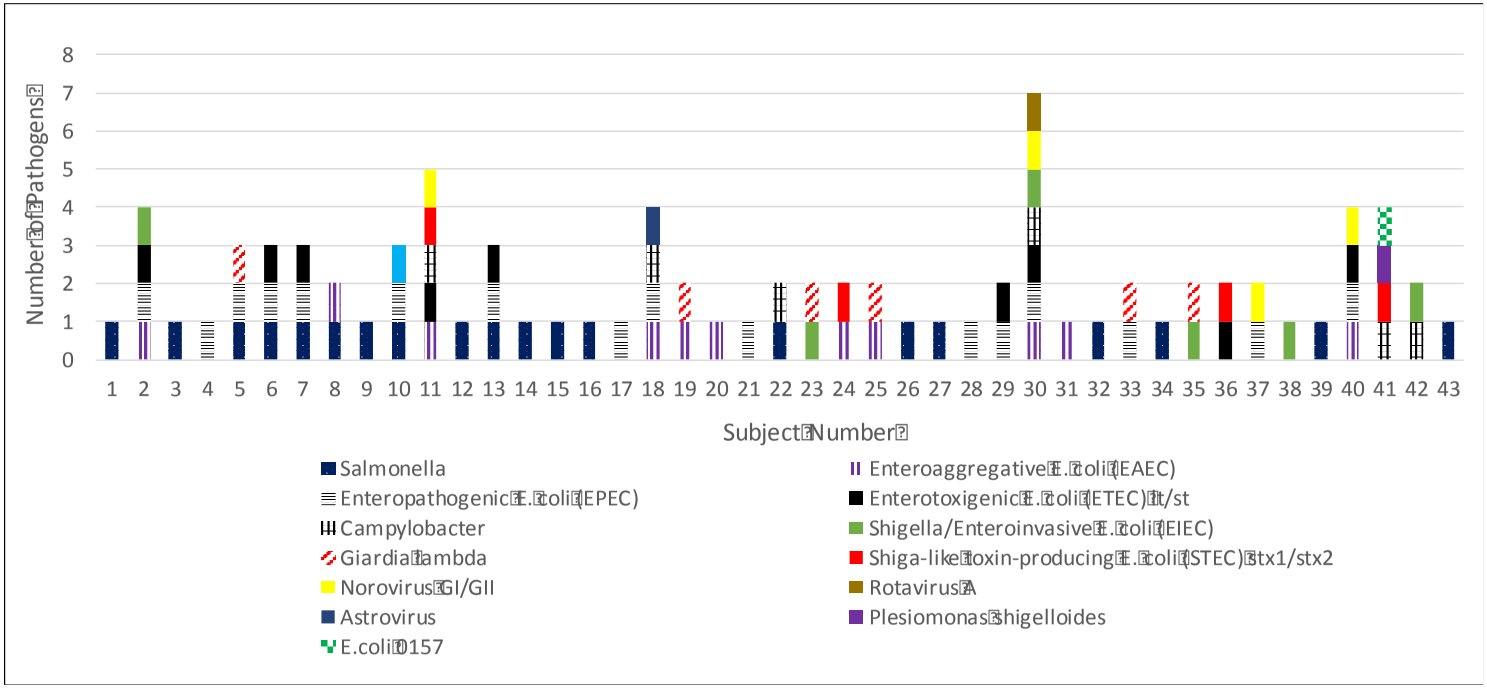


Figure 2.2 Results of multiplex PCR testing for positive stool sample

The univariate analysis of multiple demographic, clinical, and laboratory characteristics identified none that were predictive of a positive PCR result except for having a heart rate ≥ 90 beats per minute, which is likely a marker of dehydration cause by more severe diarrhea (Table 2.4). The precision of this estimate was low, however, and our small sample size limited power to detect significant effects in this analysis. Still, refining diagnostic algorithms for stool testing remains an important endeavor given the low positivity rate of traditional methods and the high cost of PCR testing [2, 24]. The presence of fecal leukocytes, for example, is often used to indicate an inflammatory process that suggests the presence of an invasive pathogen. However, neither the presence of fecal leukocytes nor threshold quantities greater than five cells per high-power field significantly increased the odds of detecting a pathogen by multiplex PCR in our study. The presence of fecal leukocytes is known to lack sensitivity since many forms of colitis occur focally [15, 16]. However, this lack of association may also reflect the high sensitivity of the PCR assay in detecting low microbe burdens.

Finally, the high proportion of subjects who received empiric antibiotics raises the potential for broadly multiplex PCR to influence antibiotic use for infectious diarrhea. Usually, empiric antibiotics for infectious diarrhea should be reserved for febrile dysentery or suspected systemic infection, severe travelers' diarrhea, or healthcare associated diarrhea suspected to be caused by *Clostridium difficile* [2, 15, 25]. In our study population of predominantly healthy young men without immune compromising conditions, 65% were empirically prescribed an antibiotic, most commonly ciprofloxacin and/or metronidazole. Receipt of either of these medications was not associated with

having a PCR result positive for any of the organisms for which these antibiotics have activity. Quinolone resistance has been documented in over 50% of Campylobacter isolates in Qatar [26] and other Gulf countries [27], and the effect of antibiotic treatment in prolonging the carrier state for nontyphoidal *Salmonella* may be particularly detrimental among our study population given the augmented risk of person-to-person transmission in dormitory settings.

Our study has certain strengths and limitations. The prospective approach of our study allowed us to ensure that all patients clinically diagnosed infectious diarrhea were offered the opportunity to enroll in the study and that formed stools were excluded from PCR testing. Retrospective studies are limited by these elements, as the likelihood of stool testing is known to vary among physicians regardless of patient characteristics [2], and laboratory protocols for stool testing may not always be strictly enforced. Various factors may have influenced the frequencies of the microbes detected in our study, including our chosen case definition, seasonal variation in the incidence of certain pathogens [26], and the unique study setting and population. Transportation to the study clinic may have also been a barrier for some migrant workers, which may have biased the pathogen distribution against those that cause more mild illness [17]. Due to an administrative issue, enrollment into our study was suspended for 19 weeks, which precluded us from reaching our planned sample size. Consequently, the precision of our estimates of the prevalence of the various pathogens was less than anticipated and we were likely underpowered to detect associations with putative risk factors. For logistical reasons, our study did not include a control group, which would have enabled us to compare the frequency of detection of pathogens, particularly the

diarrheagenic *E. coli* pathotypes, in asymptomatic persons and those with diarrhea. We also did not compare the performance of the FilmArray® PCR to traditional stool diagnostic methods such as culture, enzyme immunoassay (EIA), or uniplex PCR. Such studies have been published previously, however, and demonstrate that the FilmArray® PCR has high sensitivity and specificity in comparison to traditional methods [9, 18]. Still, the limited clinical use of stool cultures in our study precluded the determination of antibiotic susceptibility profiles and strain typing for *Salmonella* and other pathogens. Finally, although our survey was modified from the validated Minnesota Questionnaire with the input of key stakeholders at the study clinic and the Qatar Ministry of Public Health, the survey was not validated prior to being implemented for the study.

In summary, use of multiplex PCR enabled the detection of one or more pathogens in 57% of cases of infectious diarrhea among the migrant worker population in Qatar, with *Salmonella* and diarrheagenic *E. coli* the most commonly identified pathogens. Our study illustrates the utility of this diagnostic platform in epidemiologic studies and serves as a foundation for future research to understand the epidemiology and risk factors for infectious diarrhea among the migrant worker population. Further research is needed to understand the optimal use and interpretation of the FilmArray® PCR in the diagnosis of infectious diarrhea.

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APPENDIX 2

RESEARCH QUESTIONNAIRE

Research Questionnaire

Title: The Causes and Risk factors for Diarrhea among Migrant Workers in Qatar

Initial Screening Question:

Do you think you will be able to submit a sample of your stool today for the research study?

☐ **If Yes → CONTINUE with survey after stool sample is received**

☐ **If No → STOP HERE / Do NOT continue with survey**

Basic Information:

Study ID: _____

Date of interview ___ / ___ / _____

Interviewer name: _____

Language of interview: ☐ Arabic ☐ English ☐ Filipino ☐ Hindi ☐ Nepali ☐ Urdu

Location of treatment: ☐ clinic ☐ emergency room

Section I. Demographics

Please remember that you may choose not to answer questions that you feel are too personal or uncomfortable for you.

1. Age: _____ years
2. Gender: ☐ Male ☐ Female
3. What country are you from? ☐ Bangladesh ☐ Egypt ☐ India ☐ Nepal ☐ Pakistan ☐ Philippines ☐ Qatar ☐ Sudan ☐ Sri Lanka ☐ Other _____
4. Have you visited any other country during the past 21 days? Y N
   a. *If yes*, which country? _____
5. What is the name of the company you work for? _____

6. What work do you do for this company? (be as specific as possible):
   - ☐ construction worker
   - ☐ metal worker
   - ☐ crane / heavy equipment operator
   - ☐ other _____
   - ☐ driver
   - ☐ guard
   - ☐ plumber

7. When do you usually work? ☐ Day shift ☐ Night shift

8. What type of place do you live in?
   - ☐ worker camp (apartment or dormitory)
   - ☐ private apartment
   - ☐ private house

9. Do other people live in the same room with you? Y N
   a. *If yes*, how many other people? _____

10. What type of bathroom do you use at home?
    - ☐ private
    - ☐ shared with other people

Section II. History of Present Illness

1. Which did you experience first: ☐ vomit ☐ diarrhea

2. Date of onset of vomit or diarrhea (whichever occurred first): ____ / ____ / ____

1 am	7 am	13-1 pm	19-7 pm
2	8	14-2	20-8
3	9	15-3	21-9
4	10	16-4	22-10
5	11	17-5	23-11
6 am	12 noon	18-6 pm	24-12 midnight

3. Where were you when your symptoms began? ☐ home ☐ work ☐ other

4. Time of last episode of vomit or diarrhea: ____:____ AM PM
   Did you have:

Nausea	Y	N	Do not Know (DK)
Vomiting	Y	N	DK
Diarrhea	Y	N	DK

   1. *If yes:* Maximum number of stools in a 24-hour period: _____

Bloody diarrhea	Y	N	DK
Abdominal cramps	Y	N	DK

Fever	Y	N	DK
Chills	Y	N	DK
Headache	Y	N	DK
Body aches	Y	N	DK
Fatigue	Y	N	DK
Constipation	Y	N	DK
Other:_____	Y	N	DK

5. Did you take any medications (including traditional medicines or home remedies) for your current illness before you came to the clinic today?
   Y N
   a. *If yes*, what are the names of the medications?
      1. _____
      2. _____

6. Did you attend another health center before coming to the Red Crescent clinic today?
   Y N
   a. *If yes*, which one:

      a. ☐ pharmacy Date ___ / ___ / _____
      b. ☐ clinic Date ___ / ___ / _____
      c. ☐ hospital Date ___ / ___ / _____

   b. what treatment were you given? _____

7. Were you prescribed an antibiotic for any reason, prior to your current illness?
   Y N DK
   a. *If yes*, which one?
   1. _____

8. Do you know of anyone else with a similar illness during the past week?
   Y N DK
   a. *If yes*, how many other people? _____
   b. Have you had direct contact with any of those people?
      Y N DK
   c. What activities did you share with those people during the past week? (check all that apply)
      ☐ Sleep in the same room ☐ Eat in the same cafeteria
      ☐ Share the same bathroom ☐ Cook together
      ☐ Work for the same company ☐ Share food from a common plate
      ☐ Work at the same site

9. Did you have to miss work because of your illness? Y N
   a. *If yes,* how many hours? _____

10. Have you eaten any foods or drunk any beverages that you think could have caused

you to become ill? Y N DK

a. *If yes*, please describe food or drink and the date/time you had it:
_____
_____

11. Do you think you became ill from contact with someone else who was ill before you?
Y N DK

a. *If yes,* please describe when and where you contacted this person (do not use his or her name):
_____
_____

Section III. Medical History

1. How would you describe your health normally?
☐ Excellent ☐ Very good ☐ Good ☐ Fair ☐ Poor

2. Do you have any medical problems? Y N DK

☐ Diabetes	☐ Heart disease
☐ Asthma	☐ Cancer (type _____)
☐ lung disease (type_____)	☐ Liver disease (type _____)
☐ High blood pressure	☐ Other:_____
☐ Kidney disease	☐ Other:_____
☐ Anemia	☐ Other:_____

3. What medications do you take on a regular basis?
a. _____
b. _____
c. _____
d. _____
e. _____

4. Have you ever had surgery in your life? Y N DK
a. *If yes*, what surgeries did you have?
1. _____
2. _____
3. _____

5. Are you currently a smoker? Y N

6. How many times over the past 6 months have you developed a diarrheal illness?

☐ 0 times ☐ 1-2 times ☐ 3-4 times ☐ 5-6 times ☐ > 5 times

Section IV. Exposure History

1. Did you travel anywhere during the week before your illness began? Y N
   a. *If yes*, where? _____
      Dates ____ / ____ / ____ to ____ / ____ / ____

2. From what sources of water did you drink during the week before your illness began?

City tap water	Y	N	DK
Private well water	Y	N	DK
Untreated surface water (river, pond, lake)	Y	N	DK
Bottled water	Y	N	DK
Other _____			

3. Where do you usually eat the following meals? (check one box for each meal type)

Place	**Meal**		
	Breakfast	**Lunch**	**Dinner**
I do not usually eat this meal:	☐	☐	☐
food I prepare at home:	☐	☐	☐
food prepared by my roommate:	☐	☐	☐
food prepared by my co-worker: (who I do not live with)	☐	☐	☐
workplace cafeteria:	☐	☐	☐
catering company:	☐	☐	☐
restaurant:	☐	☐	☐
Other: _____	☐	☐	☐

4. Do you have a stove that works in your home? Y N

5. Do you have a refrigerator that works in your home? Y N

6. Do you cook for other people in your home? Y N

   a. What foods do you typically cook?
      _____

7. How often do you wash your hands before you eat?
   ☐ always ☐ very often ☐ sometimes ☐ not very often ☐ never

8. How often do you wash your hands before you cook?
   ☐ always ☐ very often ☐ sometimes ☐ not very often ☐ never

9. Do you drink unpasteurized (also called *raw*) milk or eat cheeses made with unpasteurized milk?

Y N DK

a. *If yes*, what types? (check all that apply)
☐ Milk ☐ Cheese ☐ Curd ☐ Cream ☐ Other_____

b. *If yes*, how frequently?
☐ Rarely (not even once a month)
☐ Monthly (at least once a month)
☐ Weekly (at least once a week)
☐ Daily

c. From what animal is the milk from? (Check all that apply)
☐ Camel ☐ Cow ☐ Goat ☐ Other _____